Proje No. 1713
Boo. No. 18002

TITLE

From Page No. 42

Split old 223 plates

Checked CHO ~~[illegible]~~ plates.

Checked Spinners

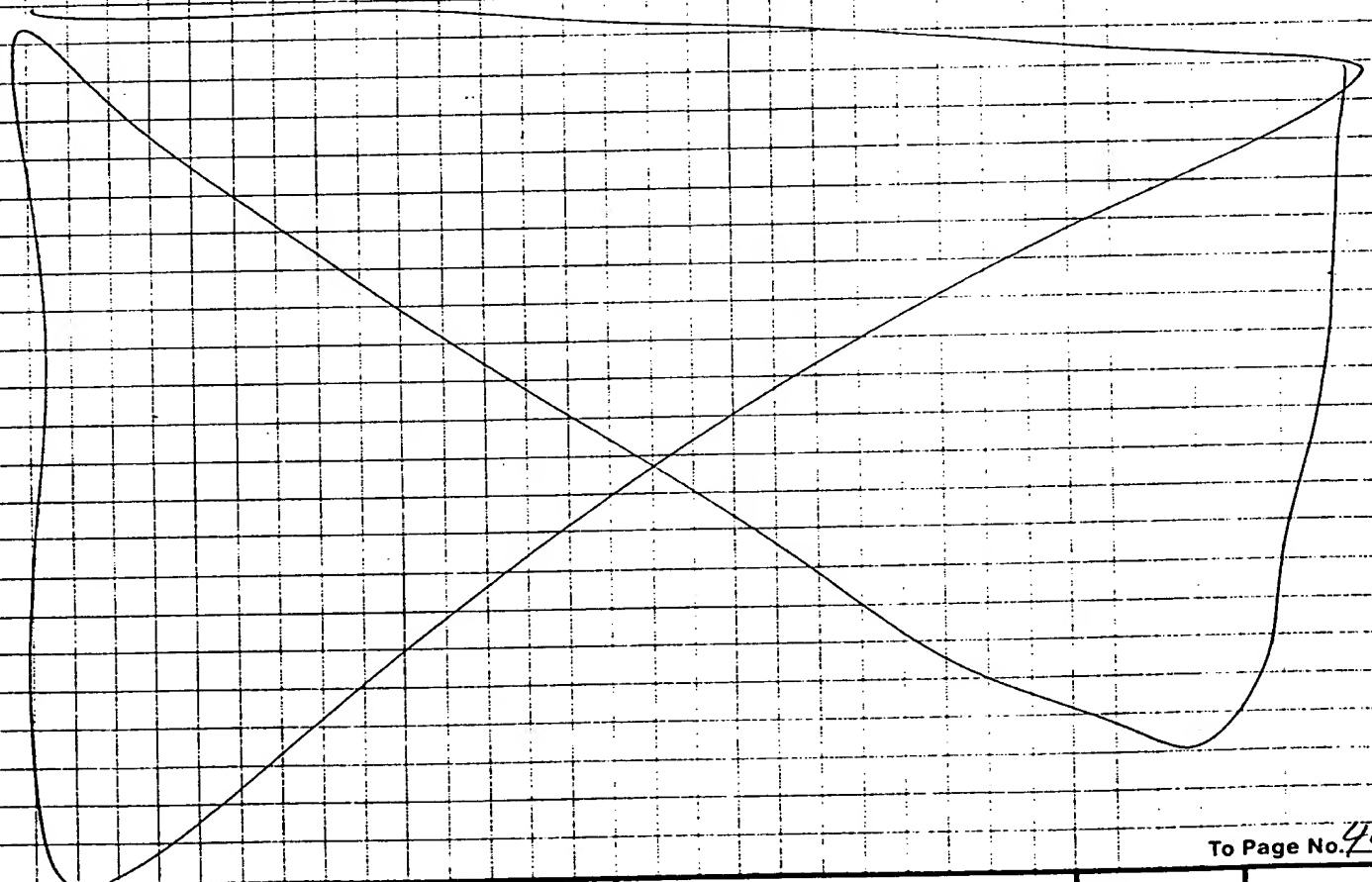
Harvested next batch of fusion protein

Run Prot A column.

Eluted → desalted → stored 4°c O/N

Concentrated fusion prot's from p. 42

Stored 4°c.



To Page No. 44

Witnessed & Understood by me,	Date	Invented by	Date TUES
		Recorded by WM Barnes	8/13/93

Project 1713

44 Book No. 18002 TITLE

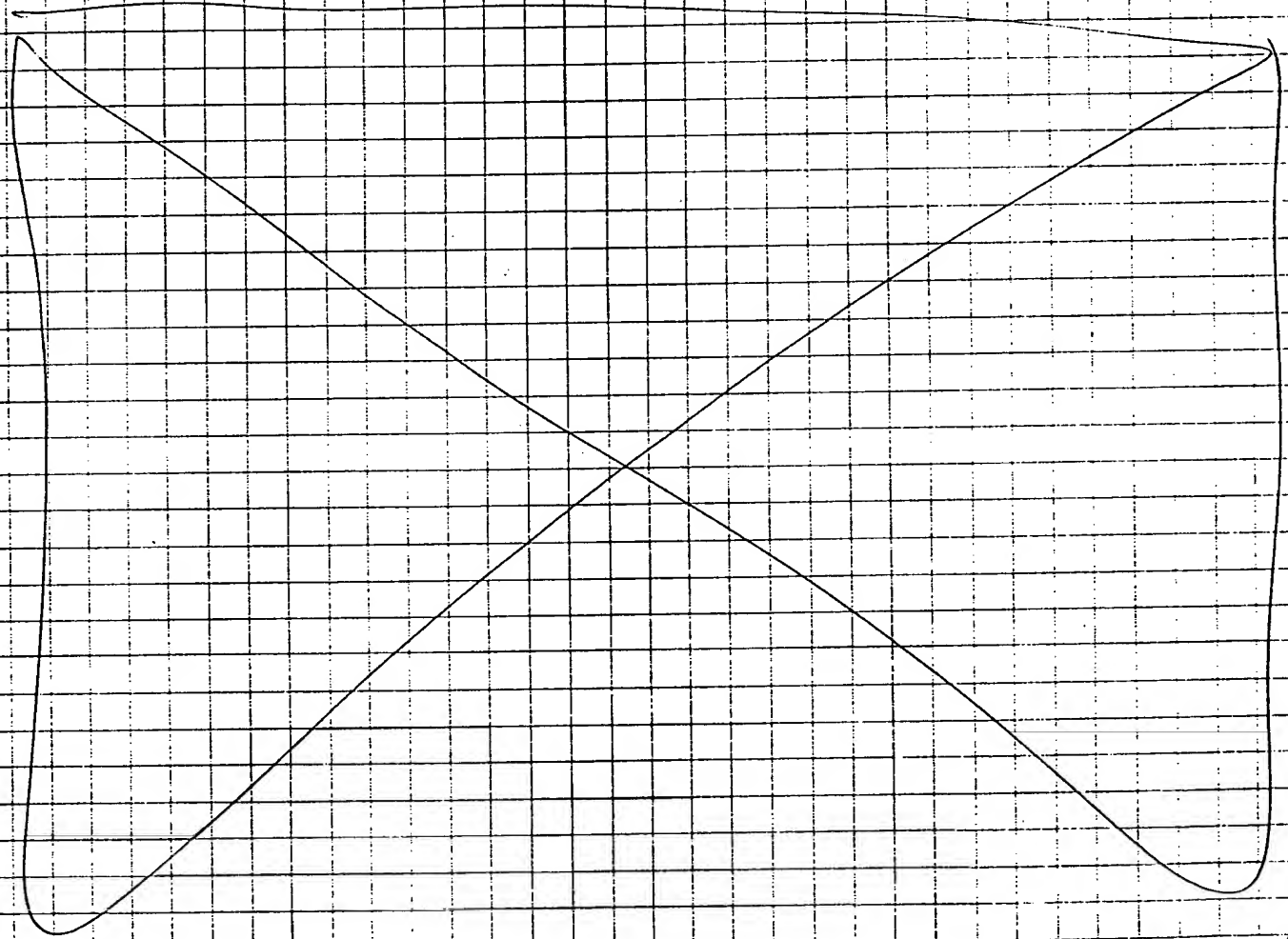
From Page No. 43

Checked all plates + spinners

Concentrated Fus prot from 8/3

Stored Both conc batches @ 4°C

Started next PSOY fusion run



To Page No. 45

Witnessed & Understood by me,	Date	Invented by	Date WED 8/4/93
		Recorded by WM Bauer	

From Page No. 44

Determined concentrations of FUSion prot. runs # (BCA

OD_{562}

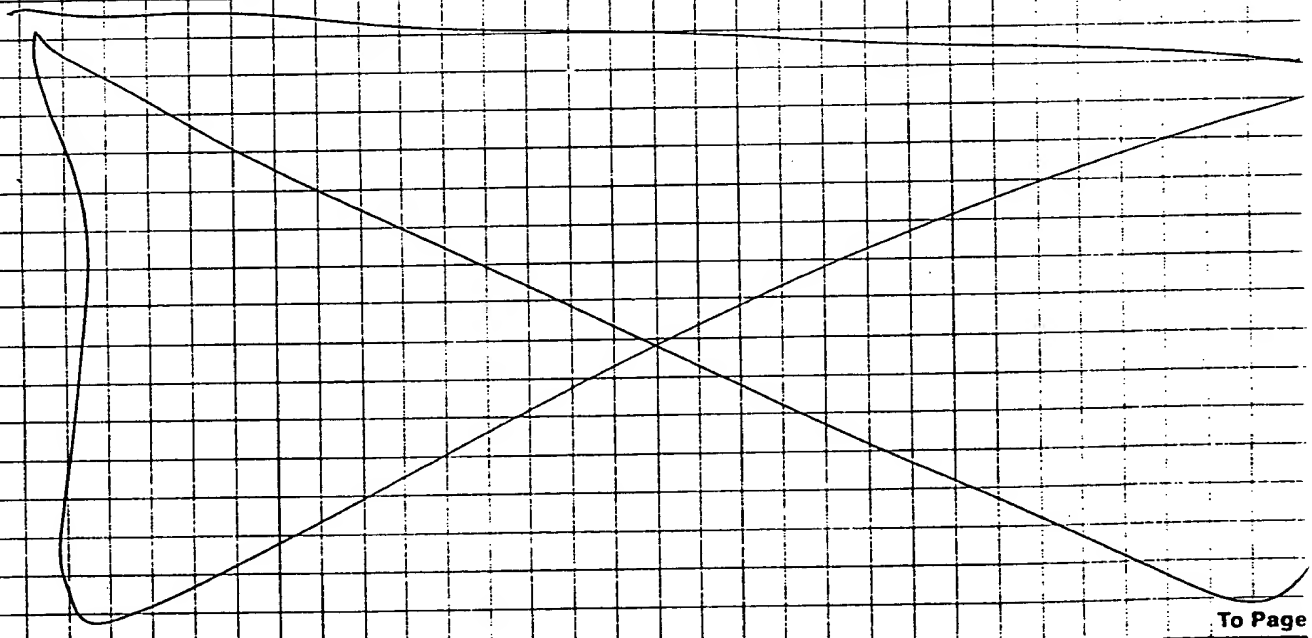
S1	=	0.190	(5μg)
S2	=	0.388	(10μg)
S3	=	0.557	(15μg)
S4	=	0.710	(20μg)
S5	=	0.851	(25μg)

#3 = 0.178 = ~4.8μg ≈ ~96 μg/ml (call it 85.

#4 = 0.364 = ~8.5μg ≈ 170 ng/ml (call it 165

see standard curve p. 46

Checked all plates & spinner → cont Inc's.



To Page

Page No. 45

Witnessed & Understood by me,

Date

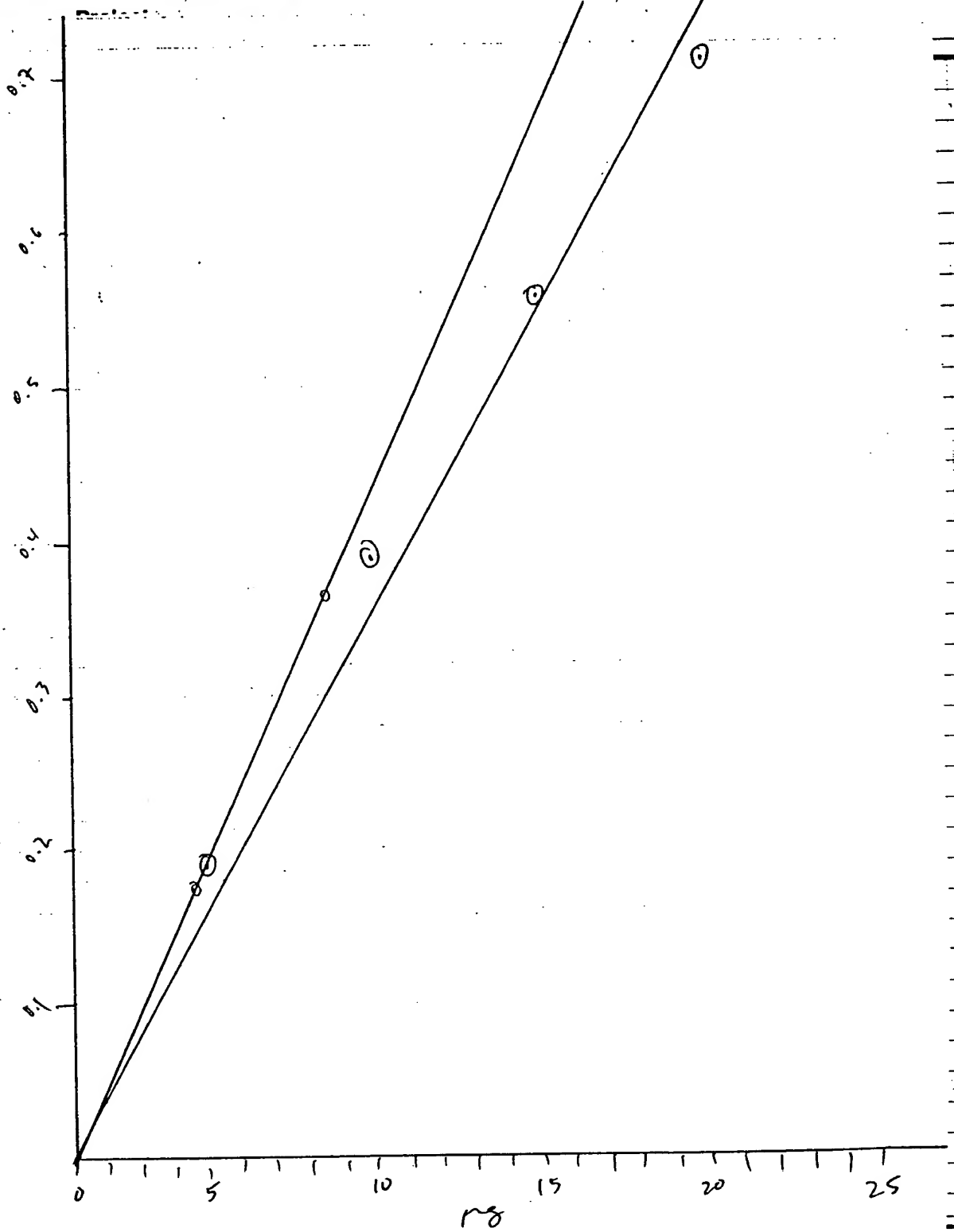
Invented by

Recorded by Will Barron

Date THURS 8/5/93

46

From Page No.



Witnessed & Understood by me,

Date

Invented by

Recorded by

Date

THURS
8/5/93

Project No. 17,
Book No. 180.

TITLE

From Page No. 96

Split all plates & spinners
Started next PSO4 run on Fus @ 11

Fus 9 spinners → ELISA DATA

		(1:10's)		Final
#1	90ng in	1.7μl =	52.9 ng/μl =	530ng/μ
#2	82ng in	2.86μl =	28.7ng/μl =	287ng/μ
#3	92ng in	5.88μl =	15.7ng/μl =	157ng/μ
#4	136ng in	3.03μl =	44.9ng/μl =	449ng/μ

These numbers do not coorespond to my BCA values. I think I will trust BCA values more than the ELISA here.

To Page No

Witnessed & Understood by me,

Date

Invented by

Recorded by Will Bacon

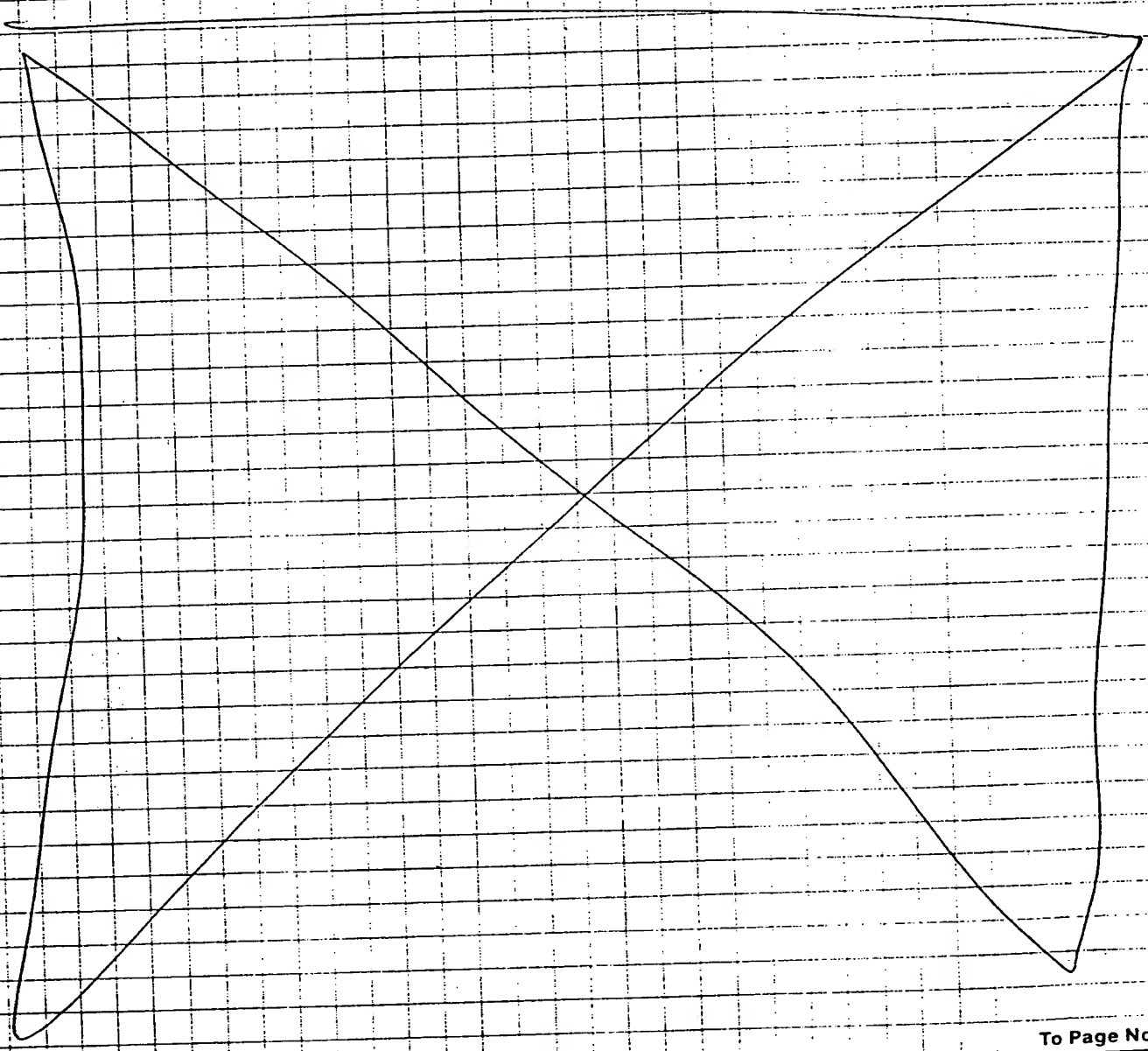
Date Fri 8/6/93

Project No. 1713
Book No. 18002 TITLE

48

From Page No. 47

Split spinners + plates
started next Fus II PS04 run.



To Page No. 49

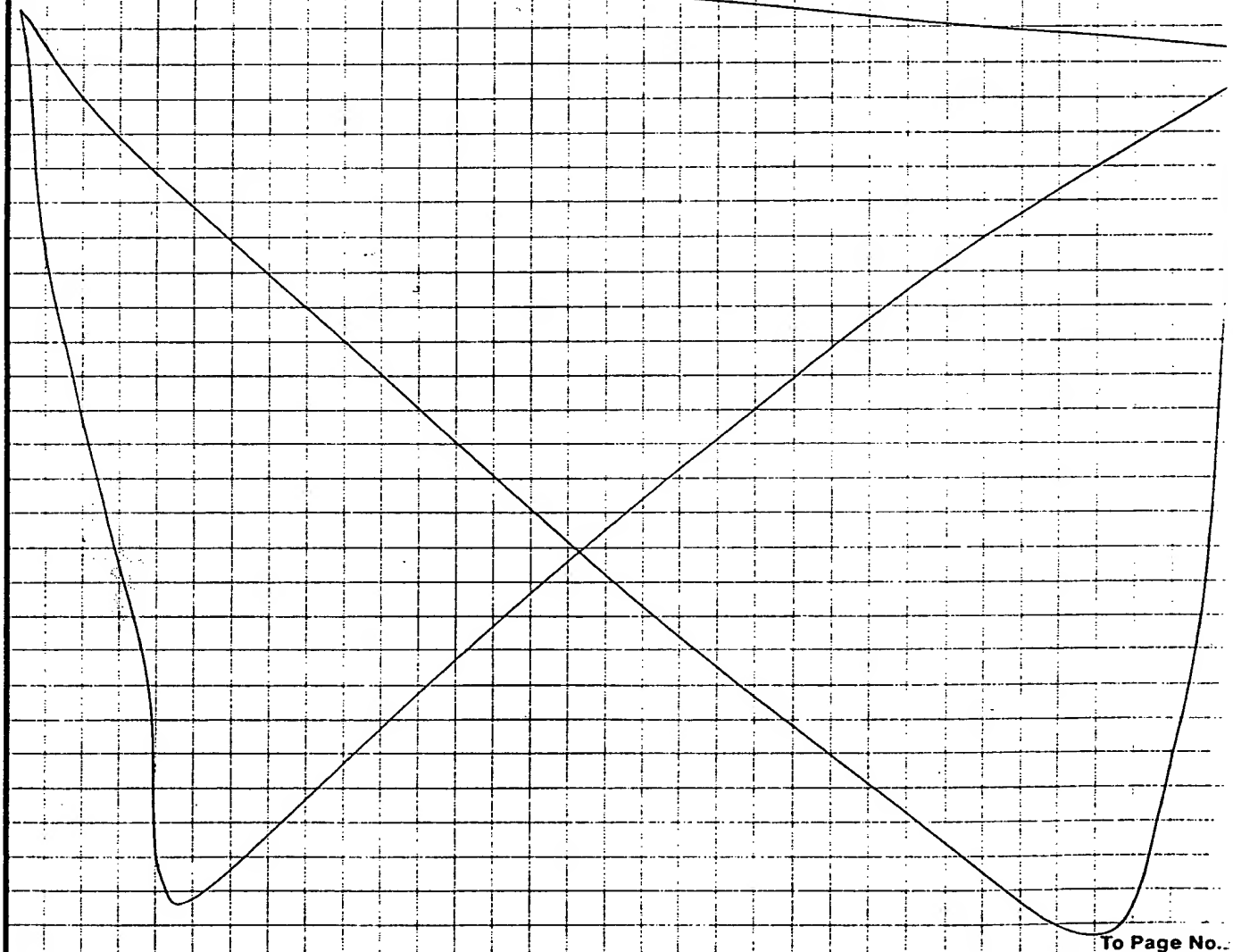
Witnessed & Understood by me,	Date	Invented by / Recorded by W.M. Barron	Date MON 8/9/93

From Page No. 98

Checked all spinners + plates

Want to try to clone mouse HPTK6 from mouse testis library (Clontech).

Started O/N C600 HF1 ⊖ in NZYDT +/- Maltose,



To Page No.

Witnessed & Understood by me,	Date	Invented by	Date TUES 8/10/93
		Recorded by WM Bacon	

Project N 713

50 Book No. 18002 TITLE

From Page No. 49

Checked plates & spinners

Harvested next PSO4 FHS II batch

Ran prot A columns

Washed, eluted, desalted (PD-10)

Stored 4°C O/N.

Titered McBell Library O/N

To Page No. 57

Witnessed & Understood by me,	Date	Invented by	Date WED
		Recorded by Wm Bacon	8/11/93

Project No.

TITLE: Book No.

From Page No. 58

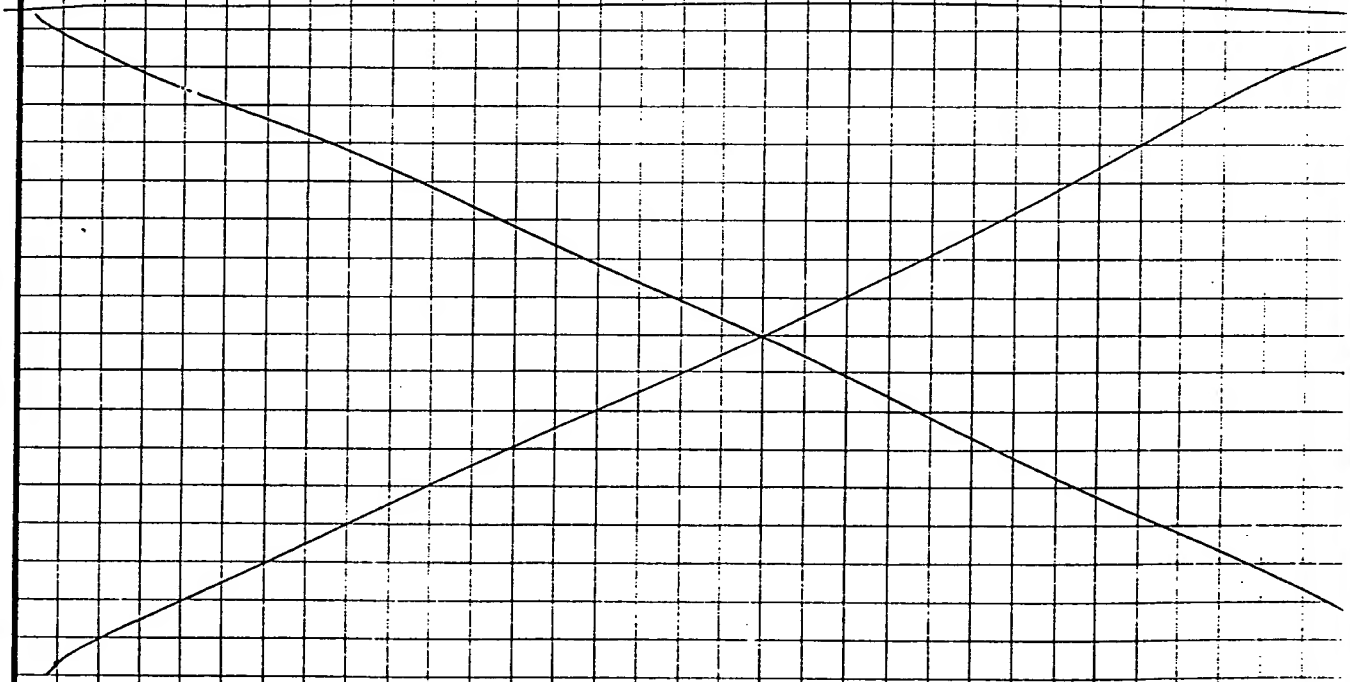
Concentrated new batch of FUS 11 → stored 4°C

Determined MinBall titer

10^{-3}	TNTC	
10^{-4}	TNTC	
10^{-5}	TNTC	
10^{-6}	TNTC	
10^{-7}	1816	$= 1.82 \times 10^{10}$
10^{-8}	292	$= 2.92 \times 10^{10}$
10^{-9}	22	$= 2.2 \times 10^{10}$
10^{-10}	2	$= 2 \times 10^{10}$

avg = 2.24×10^{10} pfu/ml

Plated out 2×10^{6} pfu total onto NZYOT O/N



To Page No.

ge No. 57

Witnessed & Understood by me,	Date	Invented by	Date THURS 8/12/93
		Recorded by WM Bardin	

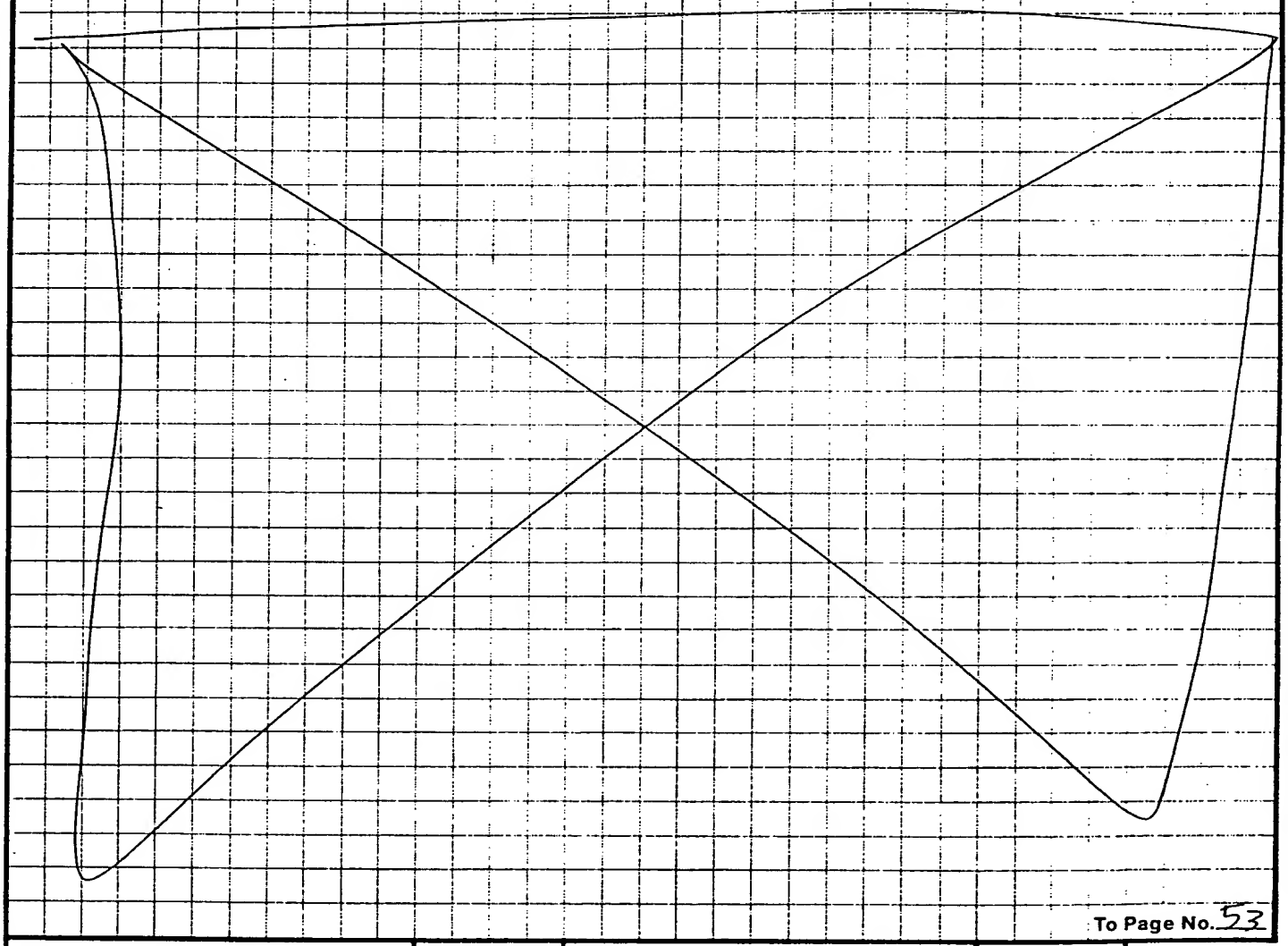
Proj. No. 1713

52 Book No. 18002 TITLE

From Page No. 51

Split all spinners & plates

Did double 1-1½ on MyBall plates
Denatured
Neutralized
Washed
UV X-linked
Baked
Stored RT



To Page No. 53

Witnessed & Understood by me,	Date	Invented by	Date FRI 8/13/93
		Recorded by Will Baron	

From Page No. 52

Prehybridized filters (mixed) in 20% formamide 42°C ~
Made probe of HPTK6 600 bp 5' end w/ random prime kit.

USER: 1 ID:32P COMMENTS:32P COUNTING
PRESET TIME: 1.00 H#: NO SAMPLE REPEATS: 1 DATA CALC: CPM
PRINTER: STD SCR:YES REPLICATES: 1 COUNT BLANK: NO
RS232: OFF RCM:YES MULTIPLIER: 1.000000

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE:YES

SAM NO	POS	TIME MIN	SCR	32P CPM	32P %ERROR	RCM	ELAPSED TIME
1	1-1	1.00	1.000	475863.44	0.29	0.00	2.02

3µl of 600

= 95,172,600

$= 9.5 \times 10^7$ cpm

Divided into 2 aliquots

Hybridized filters d/N 20% F 42°C.

Froze down all plate cells (-70°C)

Ran next prot A column on FPLC II
washed, eluted, desalted (PD-10) stored 4°C.

ge No. 53

To Page N

Witnessed & Understood by me, | Date | Invented by | Date MON
Recorded by W M Eaton 8/16/93

54 Project No. 1713
Book No. 18002 TITLE _____

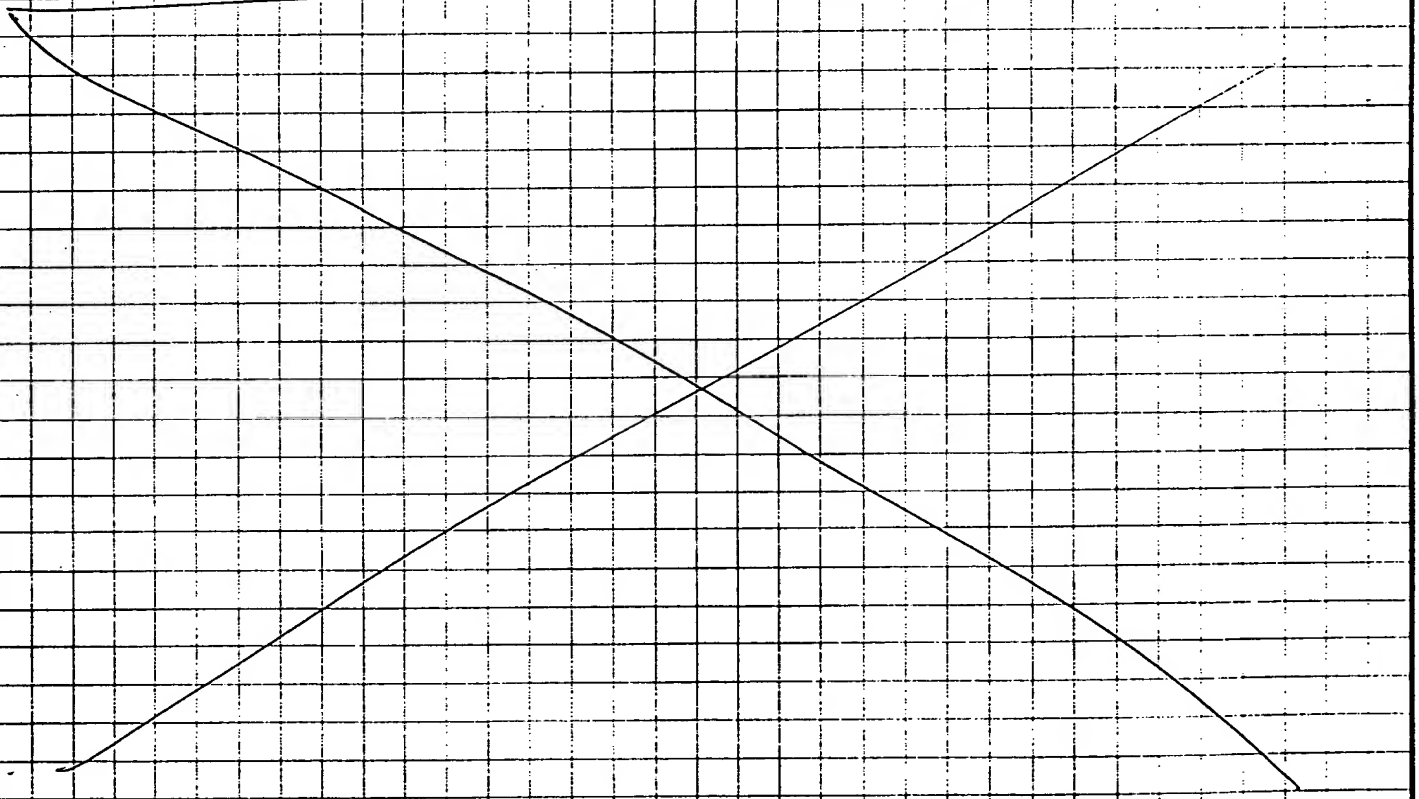
From Page No. 53

Washed O/N Mubell filters
2x / 2x SSC RT 15'
1x / 0.2x SSC 50°C 30'

Air dried, mounted, A/R'd -70°C O/N

Concentrated Fuis II @ protein from p. 53 → stored 4°C.

Split spinners



To Page No. 55

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		Thomas Barnes	THES 8/17/93

TITLE

From Page No 54

Thawed O/N cassettes → Developed

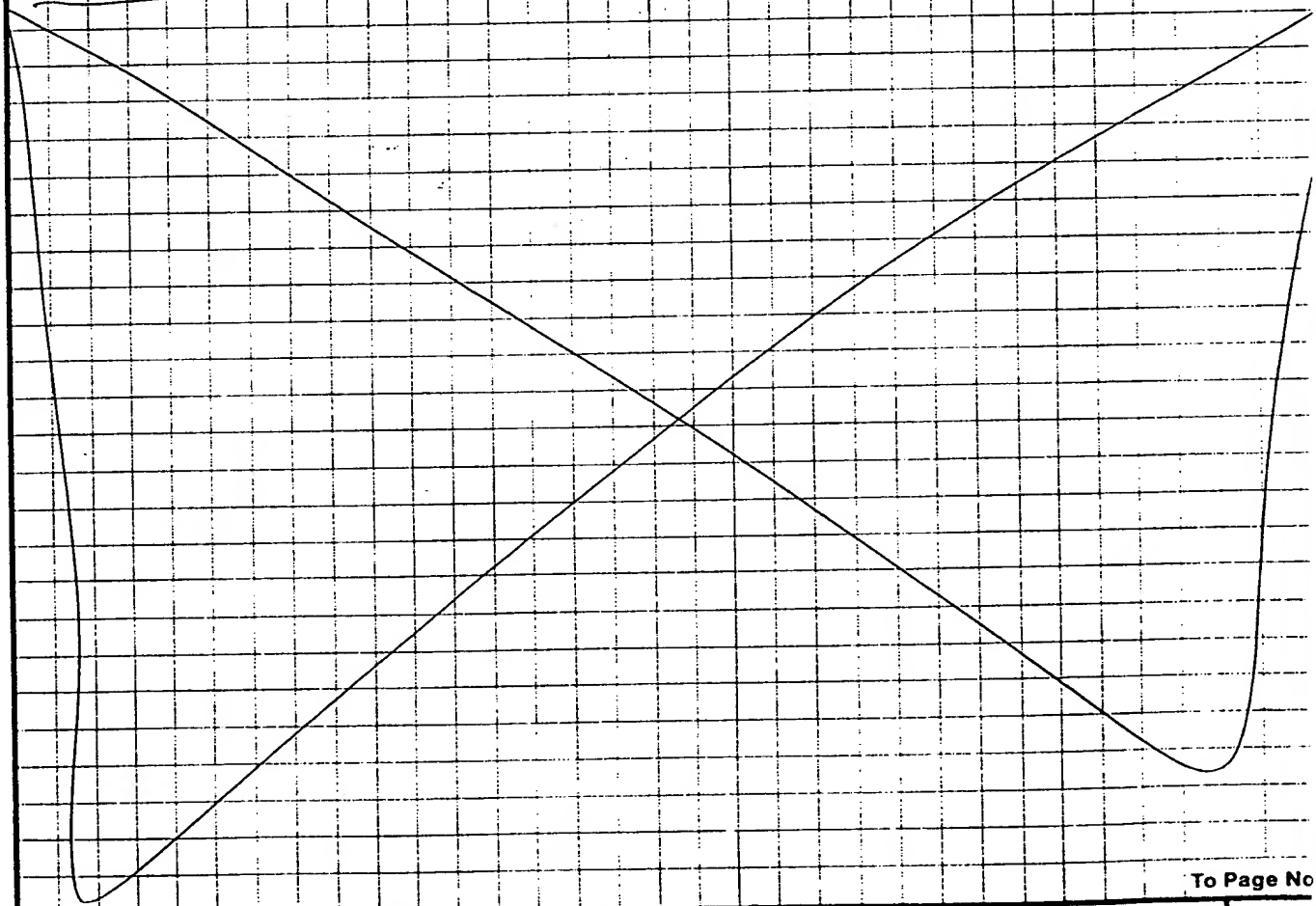
Saw 7 potential positives → picked each into 1ml PSB + $CHCl_3$

Eluted RT 4hrs

Replated each O/N @ 3 dilutions

Ran Fus 11 spinner #3 run over Prot A

Washed, eluted, desalted (PD-10) → stored 4°C



To Page No

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		W. M. Bacon	WED 8/18/93

Project J.1713

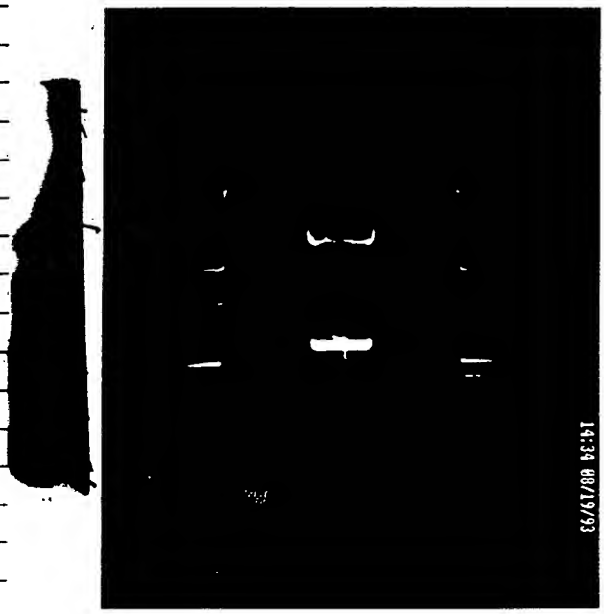
56 Book No. 18002 TITLE

From Page No. 55

Checked all spinners

Concentrated FUS11 protein from p. 55 → stored 4°C

Need to isolate HPTK6 600 bp 5' end from pGEM-3Z
Cut w/ R1 HindIII & Ran on 1% LMP



Cut out indicated Band
Did Magic PCR prep
RS'd in 200µl TE
Made probe & counted

8/19/93

USER: 1 ID:32P COMMENTS:32P COUNTING
PRESET TIME: 1.00 H#: NO SAMPLE REPEATS: 1 DATA CALC: CPM
PRINTER: STD SCR:YES REPLICATES: 1 COUNT BLANK: NO
RS232: OFF RCM:YES MULTIPLIER: 1.000000

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE:YES

SAM NO	POS	TIME MIN	SCR	32P CPM	32P %ERROR	RCM	ELAPSED TIME
1	1-1	1.00	1.000	73817.22	0.74	0.00	1.50

1% = 7,381,700 ?

Page No. 57

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		Will Bacon	8/19/93

TITLE

From Page No. 56

Did lifts on all 21 O/N rescreens of MuBall/TK6
Prehyb'd 20°w F 42°C 4 hrs
Hyb'd O/N w/ fresh probe 42°C.

Ordered primers to do 2hybrid system on HPTK6

Run PCR's O/N
Primers sets
1) HPTK6 #1 & WB1 (tyro P12)
2) HPTK6 #3 & WB1 (tyro P12)
3) HPTK6 #2 & tyro P13
4) HPTK6 #2 & tyro P16

10 µl buffer
16 µl dNTP's
1 µl 1st primers
1 µl 2nd primers
2 µl 1:10 pRK5/TK6
2 µl Taq
69 µl H_2O
100
+ 100 µl oil

Conds

94°C 5'
55°C 30" 1 cycle
72°C 30"

98°C 30"
55°C 30" 4 cycles
72°C 30"

96°C 30"
55°C 30" 20 cycles
72°C 30" w/ 1 sec auto-ext

72°C 10' 1 cycle

4°C Soak

Ran O/N

To Page No. 57

Witnessed & Understood by me,

Date

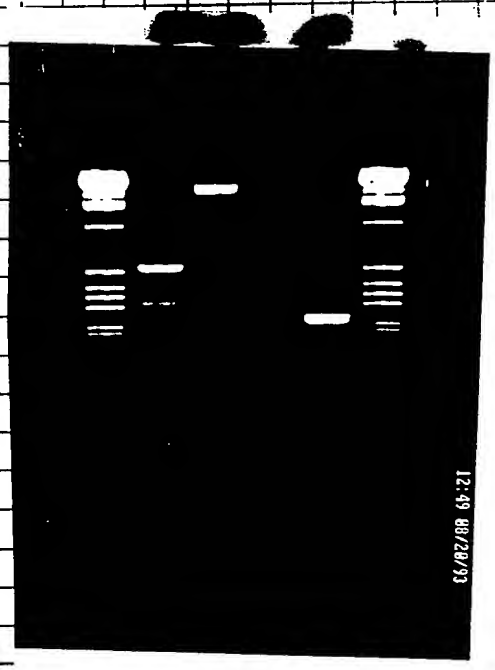
Invented by

Recorded by SMM Bacon

Date THURS 8/19/93

From Page No. 57

Extracted O/N PCR's 1x 100µl CHCl3
Ran 10µl each on gel



Did Magic PCR preps
Stored -20°C

Checked spinners → count

Washed O/N MuBall filters
2x/2x SSC RT 15'
1x/0.2x SSC 50°C 30'
Air dry, mounted
A/R'd -70°C O/N.

To Page No. 59

Witnessed & Understood by me,	Date	Invented by Will Bacon	Date FRI 8/20/93
		Recorded by	

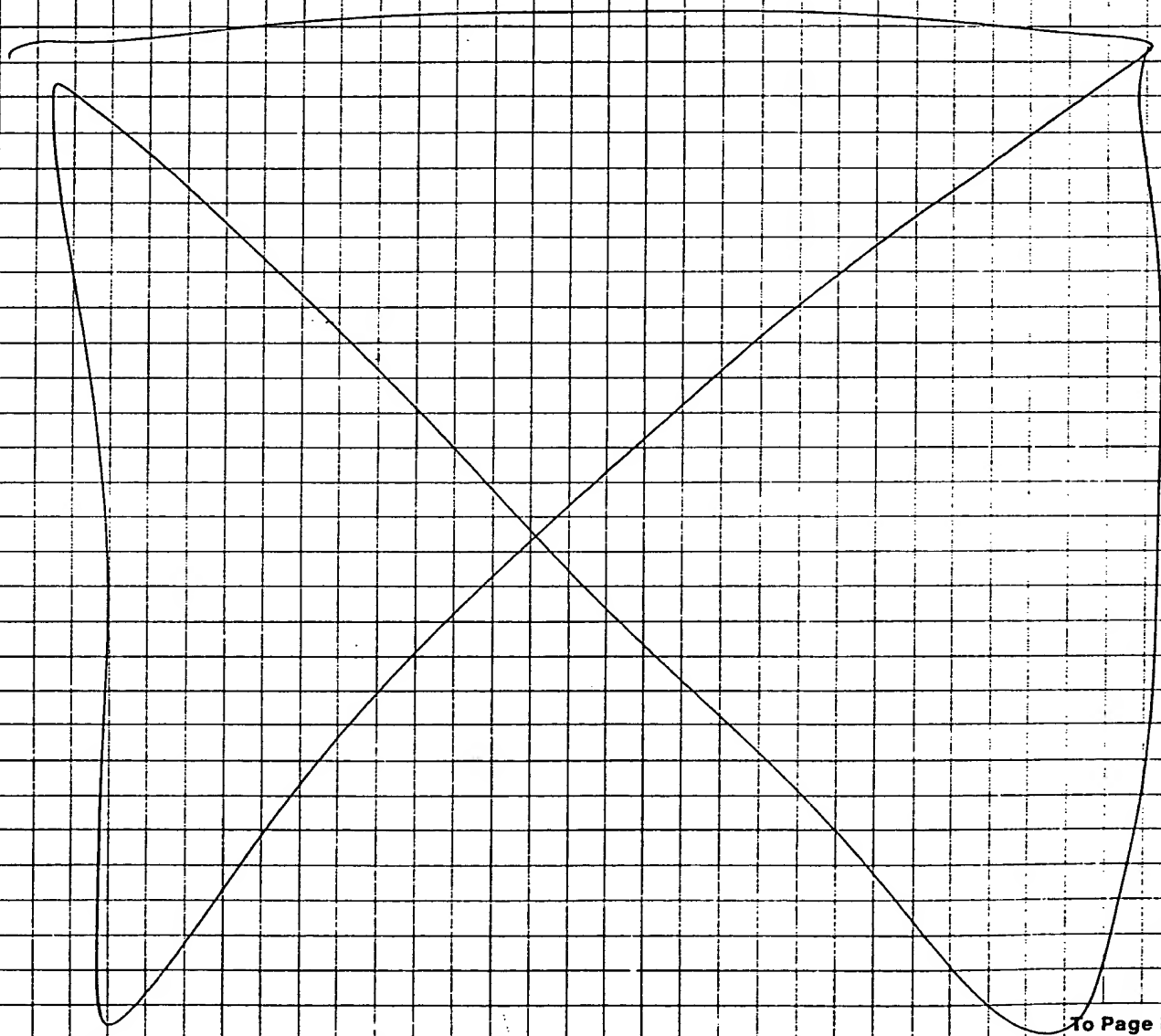
TITLE _____ Project No. 1 Book No. 1

From Page No. 58

Developed o/n A/E's

No 2° positives seen.

I will replate library later.



To Page No.

Witnessed & Understood by me,	Date	Invented by	Date SAT
		Recorded by WM Bacon	8/21/93

No. 59

Project No. 1713
60 Book No. 18002 TITLE

From Page No. 59

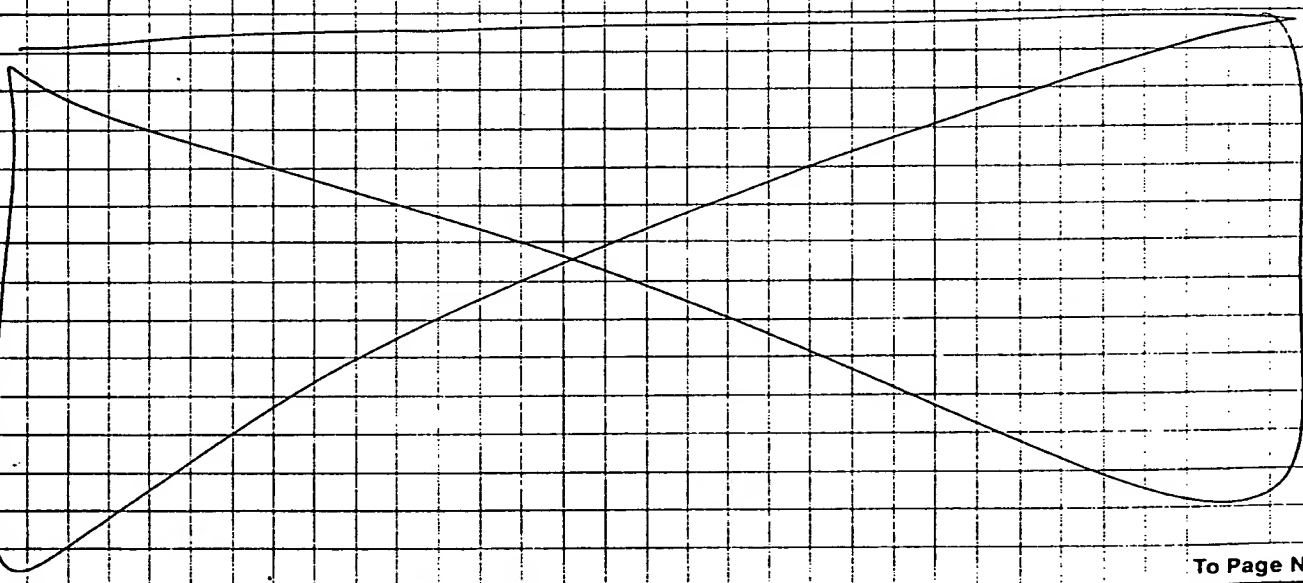
Ran Prot A column on last Fus V spinner PS04.
Washed, eluted, desalted (20mM) → stored 4°C.

split spinners

Ran O/N SDS (10%) gel on all Fusion batches

Fus 9.2
9.3
9.4
11.1
11.2
11.3
} 5µg each
} conc not known so ran 12µl each.

(Not 11.4 → not concentrated yet)



To Page No. 61

Witnessed & Understood by me,	Date	Invented by Will Bason	Date Mon 8/23/93
		Recorded by	

TITLE

From Page No. 60

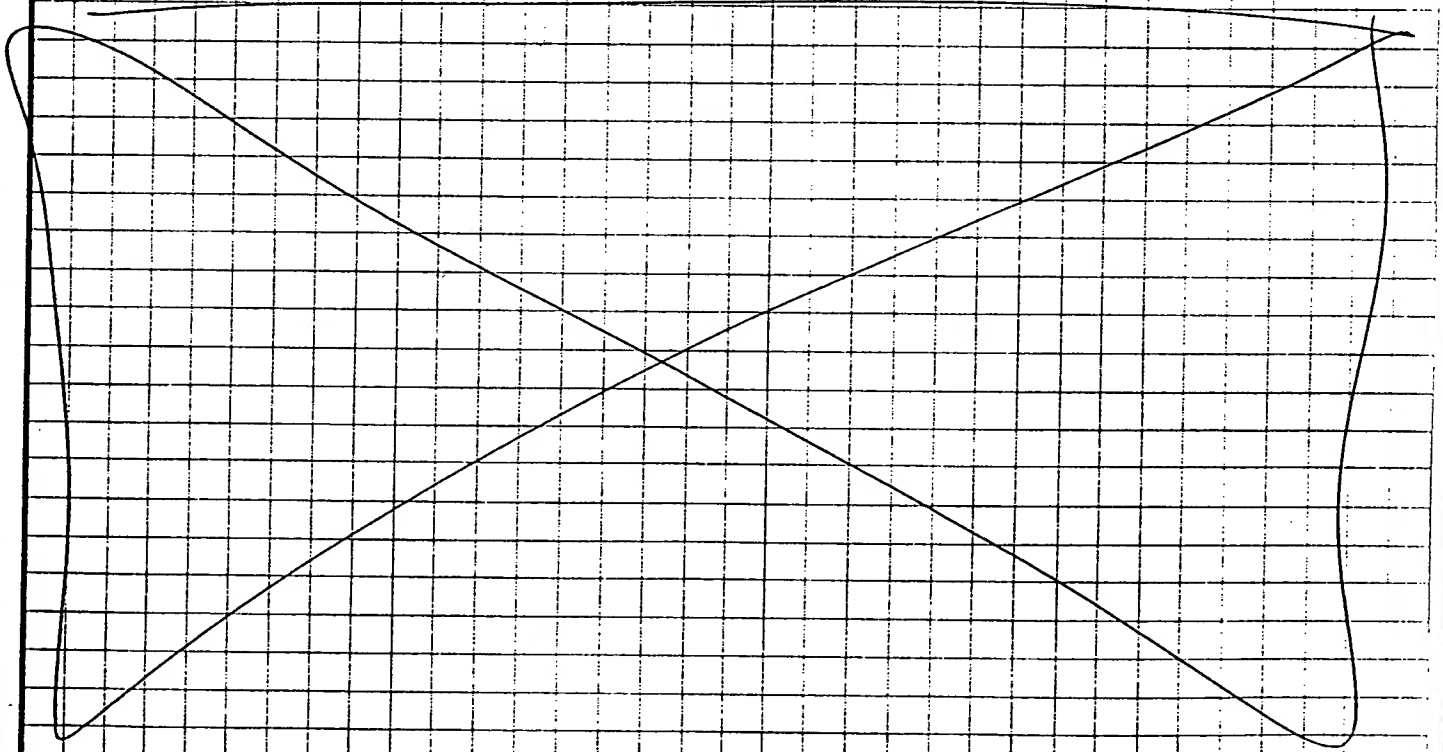
Stopped O/N SDS gel

Fixed, coomassie stained, destained while on vacation until 9/8/93.

concentrated FUS II #4

Stored 4°C.

KPB is in charge of spinners while I'm gone.



To Page No. 62

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date TUES 8/24/93

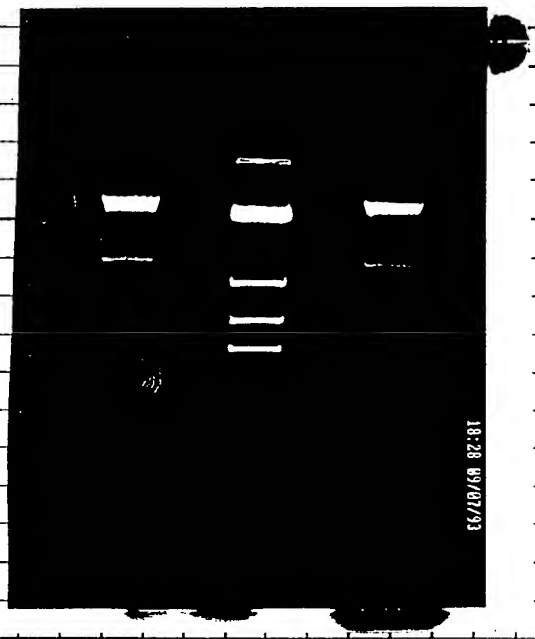
62 Project No. 1713 Book No. 18002 TITLE

From Page No. 61

Checked spinners

Want to re-probe MiniBall Library w/ TK6 transmembrane piece.

Cut pRK5/TK6 w/ AvrII/HindIII → 1% LMP



Isolated indicated band

Did Magic PCR prep.

Stored -20°C.

To Page No. 63

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by Will Bacon	9/7/93

TITLE:

From Page No. 62

Prehybd Mubell filters 20% F 42°C ~4hrs
Labelled a double batch of transmem. probe → coun

USER: 1 ID:32P COMMENTS:32P COUNTING
PRESET TIME: 1.00 H#: NO SAMPLE REPEATS: 1 DATA CALC: CF
PRINTER: STD SCR:YES REPLICATES: 1 COUNT BLANK: NO
RS232: OFF RCM:YES MULTIPLIER: 1.000000

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE:YES

SAM NO	POS	TIME MIN	SCR	32P CPM	32P %ERROR	RCM	ELAPSED TIME
1	1-1	1.00	1.000	52689.55	0.87	0.00	1.48
2	1-2	1.00	1.000	67030.16	0.77	0.00	2.89

1 ×100 = 5,268,900

2 ×100 = 6,703,000

11,971,900

× 100 ml each = 119,719 counts/ml

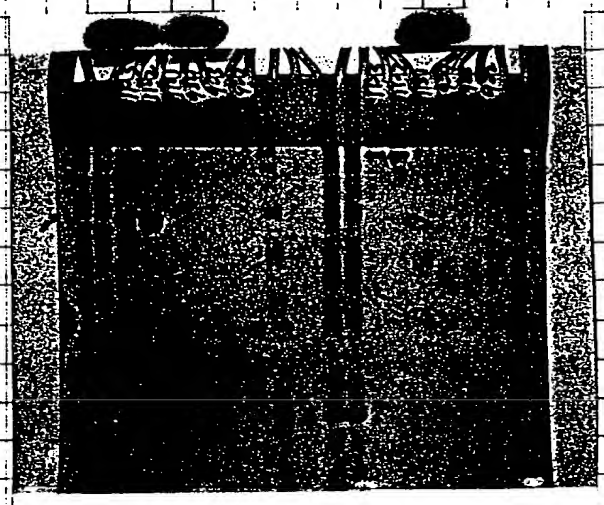
Labelling rxns are not working very well.
Hybrid 42°C O/N 20% F.

To Page No. 63

Witnessed & Understood by me,	Date	Invented by / Recorded by Will Bacon	Date WED 9/8/93

From Page No. 63

Photographed destained SDS gels of Fus Protein from ~8/4



This looks like my protein is totally degrading

Ran a fresh gel O/N on all samples

Spinner flasks are really looking bad. Media is sort of purple → added fresh media to them

Cont Inc

To Page No. 65

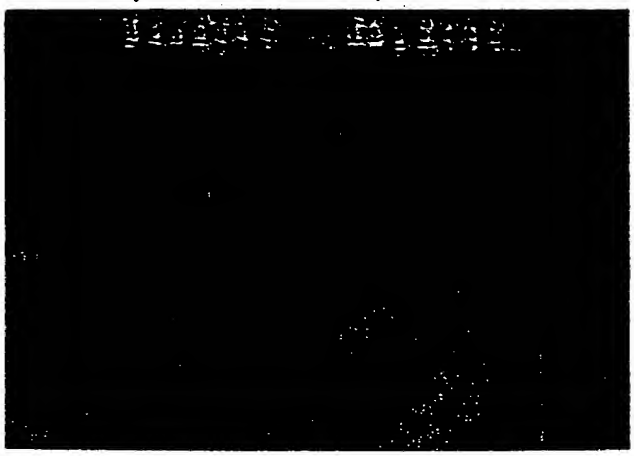
Witnessed & Understood by me,	Date	Invented by / Recorded by Will Barron	Date WED 9/8/93

Subject No. 17
Book No. 18

TITLE

From Page No 64

Stopped O/N SDS gel → Fixed, stained, de-stained, photograph



HORRIFYING!

Something has happened to all of my fusion protein.

I checked the tube more closely. I think that everything has aggregated.

I will try to re dissolve it later.

Spinner flasks look like shit. They may be de Added HEPES to both.

Want to see if I can make TK6 probe w/ PCR

Ran test PCR's

Primers:

1)	Tyro	P13/P23
2)		P16/P23
3)		P13/P24
4)		P16/P24

To Page No.

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date THURS 9/9/93

66 Project No. 1713 Book No. 18002 TITLE

From Page No 65

10 µl 10× buffer
16 µl dNTP's
1 µl 1st primer
1 µl 2nd primer
2 µl 1:10 PRKS/TKL
1 µl Taq
69 µl H_2O
100
+100 µl O.I

Conds

94°C 5'
55°C 30"
72°C 1'
1 cycle

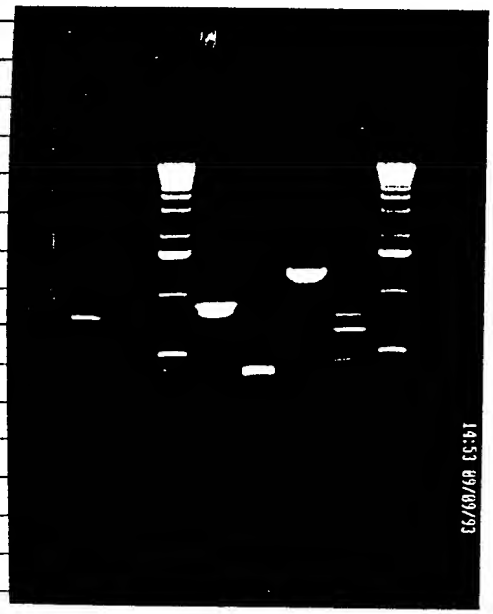
98°C 30"
55°C 30"
72°C 1'
4 cycles

96°C 30"
55°C 30"
72°C 1' w/ 1 sec auto ext
25 cycles

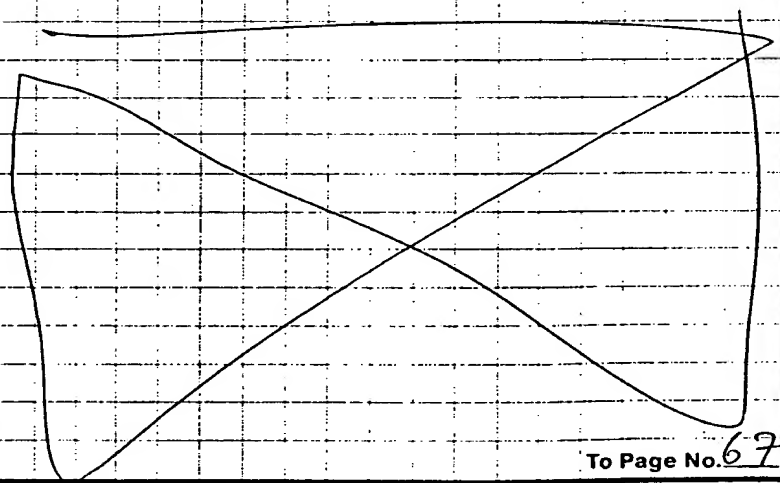
72°C 10'
1 cycle

4°C soak

Extracted each 1× 100 µl $CHCl_3$
Ran 10 µl each on gel (1%)



Use either 13/24 or 16/24 for probe



To Page No. 67

Witnessed & Understood by me,	Date	Invented by W. M. Bauer	Date THURS 9/9/93
		Recorded by	

TITLE

From Page No. 66

Ran 13/24 + 16/24 primer sets

Probe RCR
10µl buffer
16µl dNTP's (No dCTP!)
2µl α^{32}P-dCTP
1µl 1st primer
1µl 2nd primer
2µl 1:10 pRK5/TK6
1µl Taq
49µl H2O
100
+ 100µl Oil

Same conds as on p. 66

Purified Through G50 superfine
Counted

USER: 1 ID:32P COMMENTS:32P COUNTING
PRESET TIME: 1.00 H#: NO SAMPLE REPEATS: 1 DATA CALC:
PRINTER: STD SCR:YES REPLICATES: 1 COUNT BLANK:
RS232: OFF RCM:YES MULTIPLIER: 1.000000

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE:YES

SAM NO	POS	TIME MIN	SCR	32P CPM	32P %ERROR	RCM	ELAPSED TIME	
1	1-1	1.00	1.000	514575.72	0.28	0.00	2.11	13/24
2	1-2	1.00	1.000	58166.70	0.83	0.00	3.52	16/24

Denatured Both (combined) + added to already hybs.
Rubball plates
Count Turned 42°C O/N.

Page No. 67

To Page

Witnessed & Understood by me,

Date

Invented by

Recorded by

Date Hours
9/9/93

From Page No. 67

Transferred non-aggregated Fus Prot to B. Fendly

Washed O/N Hyb'ing MuBall filters 2x/2x SSC RT 15'
1x/1x SSC 50°C 30'

Air dried, mounted, H/R'd -70°C O/N

Tried dissolving aggregate in various pH (6.7 → 5.8) of phosphate buffer → no good.

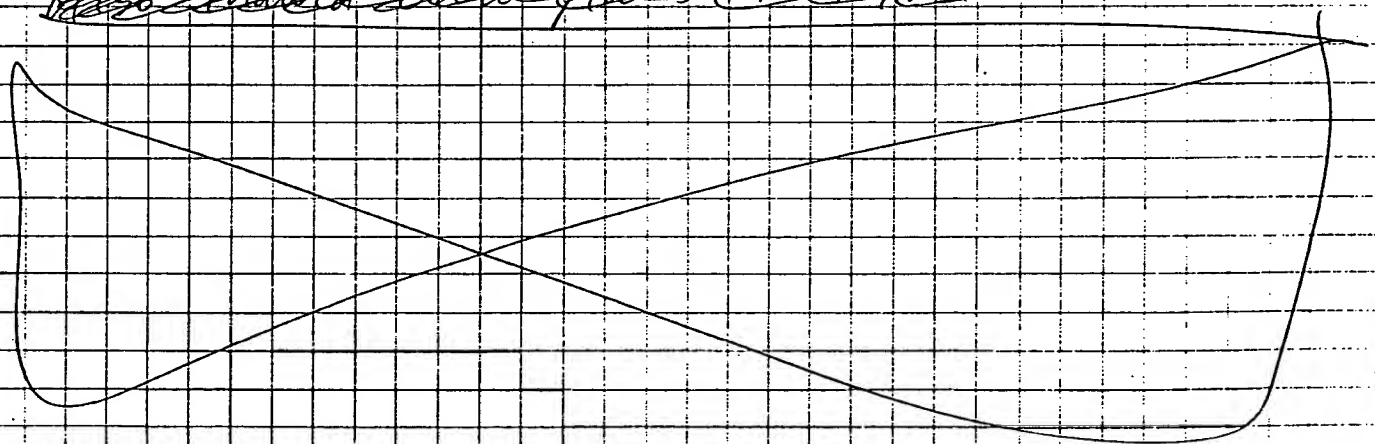
Tried Boiling in SDS → nothing
" Boiling in glacial acetic acid → nothing

The shit is history.

Meanwhile spinner flasks are definately dead!

Thawed FUS 9 & 11 each ~~[illegible]~~ ~~directly from spinners~~ onto 10 cm dishes.

~~Also thawed onto plates (FUS 9 & 11~~



To Page No. 69

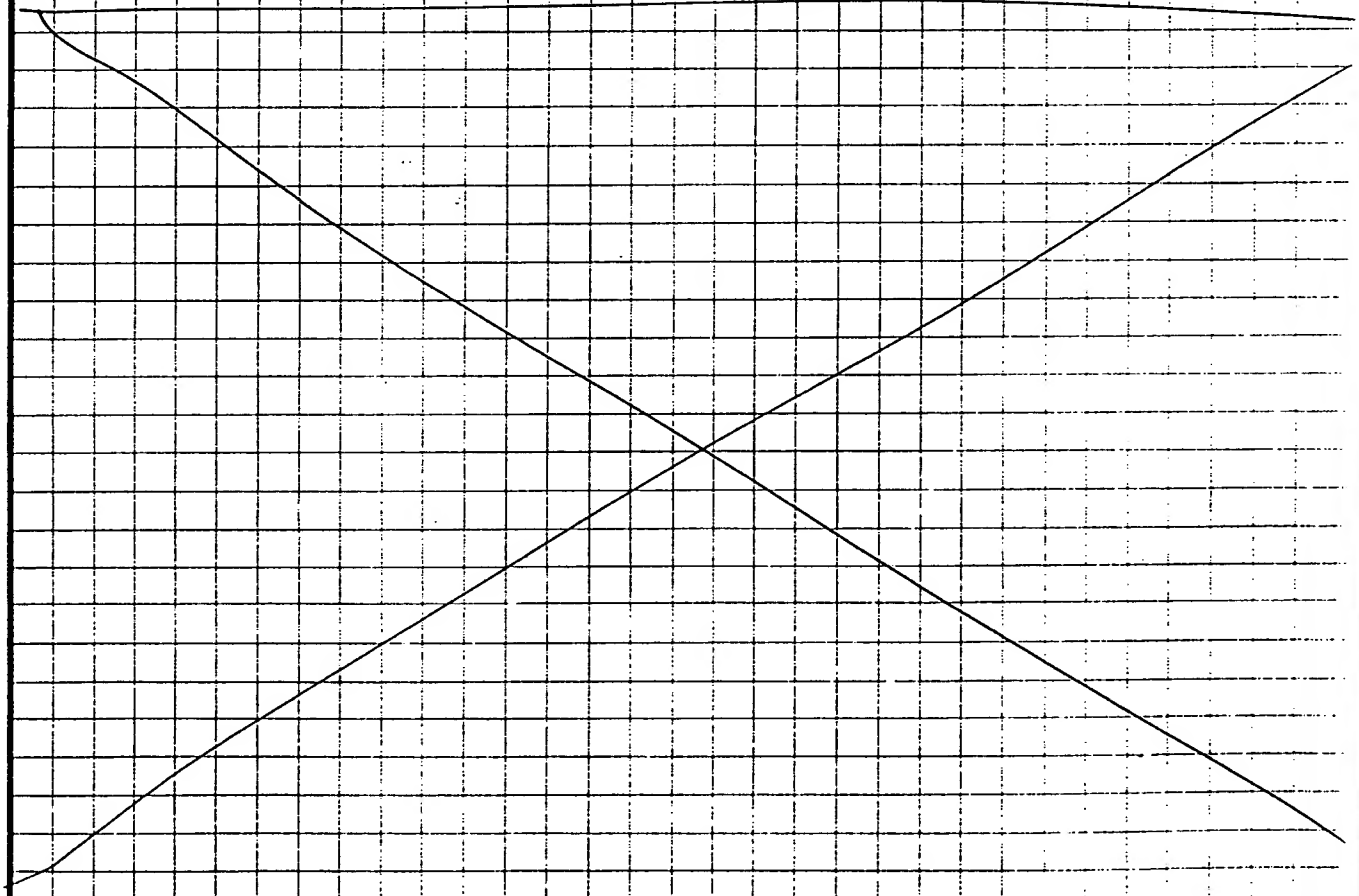
Witnessed & Understood by me,	Date	Invented by	Date FRI
		Recorded by WM Bacon	9/10/93

From Page No. 68

Thawed O/N cassettes → developed. Stored RT.

Even after boiling aggregated protein O/N it has not dissolved in SDS or HOAc.

8) Checked FUS 9 + 11 cells. Looking good.
Washed + changed media on both.



To Page No

Witnessed & Understood by me,	Date	Invented by	Date 9/11/93
		Recorded by W.W. Bauer	

No. 69

Project No. 1713

70 Book No. 18002 TITLE

From Page No. 69

Split Fus 11 + 9 into 250ml spinners.

Checked A/R's from SAT → no positives observed.
Made new PCR probe as on p. 67
purified + counted

USER: 1 ID:32P COMMENTS:32P COUNTING
PRESET TIME: 1.00 H#: NO SAMPLE REPEATS: 1 DATA CALC: CP
PRINTER: STD SCR:YES REPLICATES: 1 COUNT BLANK: NO
RS232: OFF RCM:YES MULTIPLIER: 1.000000

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE:YES

SAM NO	POS	TIME MIN	SCR	32P CPM	32P %ERROR	RCM	ELAPSED TIME
1	1-1	1.00	1.000	321280.75	0.35	0.00	1.70
2	1-2	1.00	1.000	208851.97	0.44	0.00	3.20

0.5% each

A = 321,280 × 2 = 642,560 × 100 = 64,256,000

B = 208,851 × 2 = 417,702 × 100 = 41,770,200

106,026,200

Re hyb'd filters O/N. 42°C 20%F

To Page No. 71

Witnessed & Understood by me,	Date	Invented by	Date MON
		Recorded by Will Barton	9/13/93

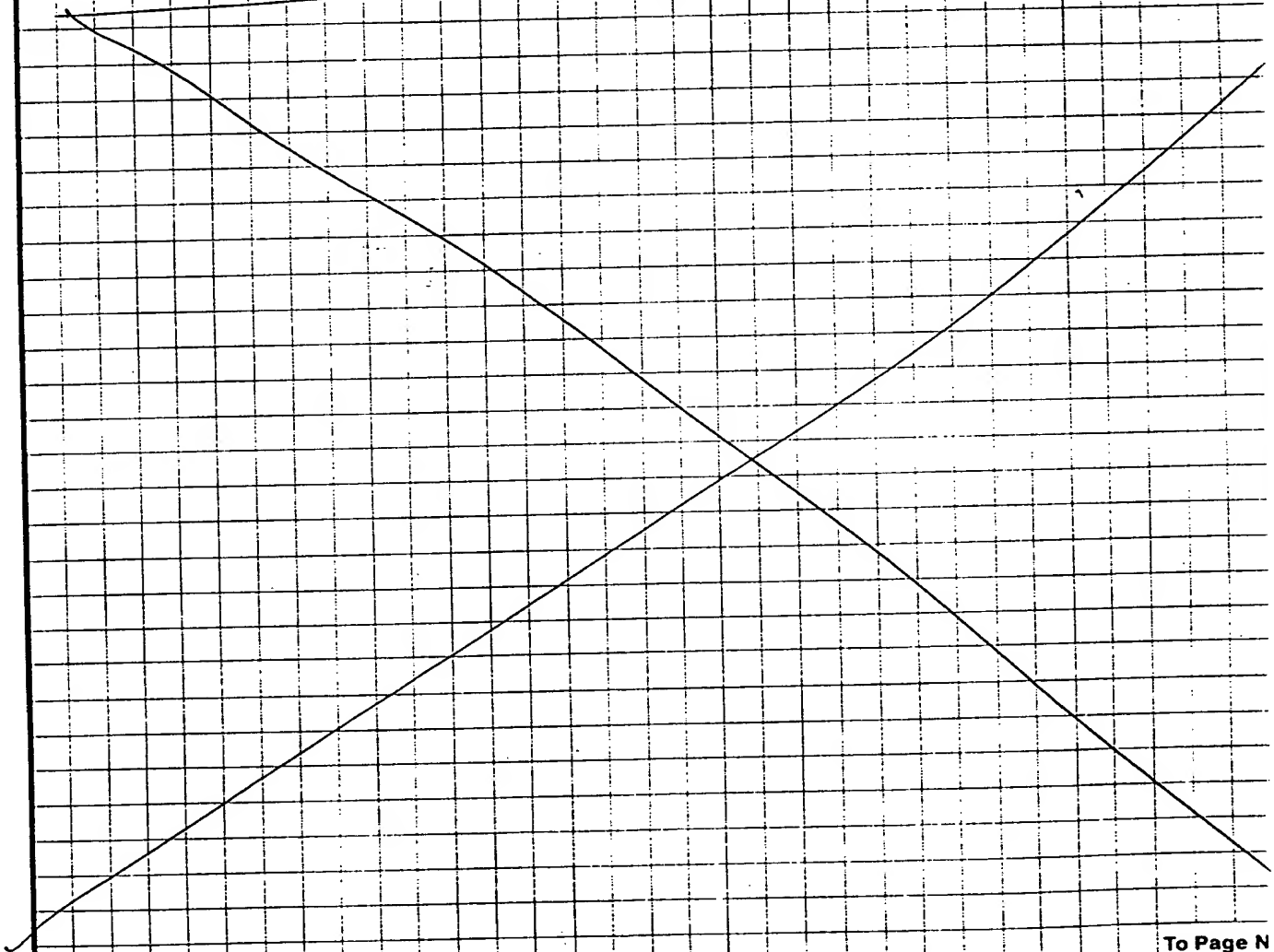
TITLE

From Page No. 70

Checked spinners + plates → split plates 1:10
Split spinners 1:2 into 500 ml each.
(scale up).

Washed o/n filters 2x/2xSSC RT 15'
0.1x/1xSSC 50°C 30'

Air dried, mounted A/R'd -20°C o/n.



To Page N

e No. 71

Witnessed & Understood by me,	Date	Invented by / Recorded by J.M.Barron	Date TUES 9/14/93

Project No. 1713

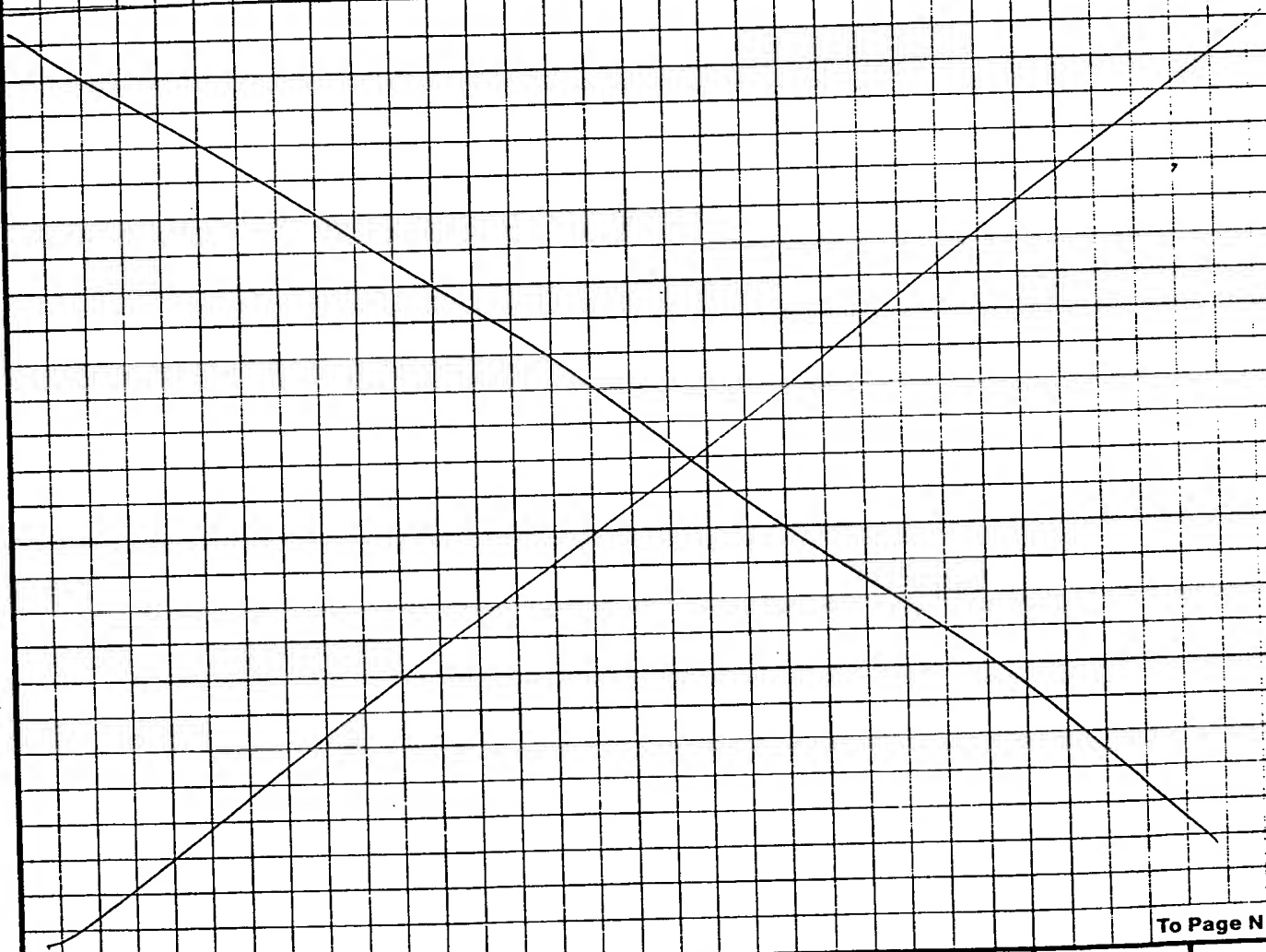
72 Book No. 18002 TITLE

From Page No. 71

Developed o/w A/R's → made picks (13 total)

Re plated o/w.

Checked spinners + plates



To Page No. 93

Witnessed & Understood by me,	Date	Invented by Will Bolton	Date WED 9/15/93
		Recorded by	

TITLE

From Page No. 72

Checked spinner → split each to 2 x 1L (Fus 9 & 11)
Checked plates → cont Inc.

Did Lifts on MiniBall 1° pick replates (2 0's)
Denatured, neutralized, washed, x-linked, Baked

PRE-Hyb'd in 20% F 42°c ~6 hrs
made transmembrane probe as described via PCR
purified & counted

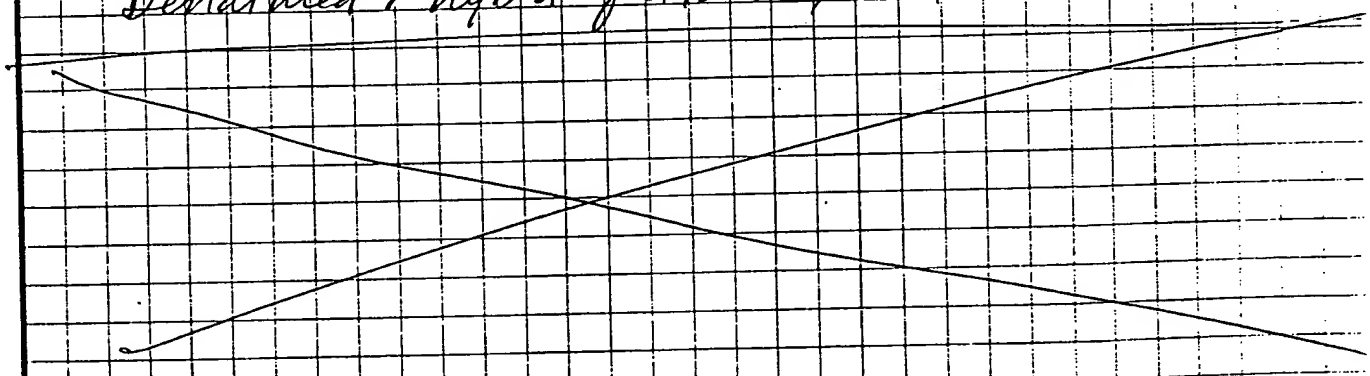
USER: ● ID:32P COMMENTS:32P COUNTING
PRESET TIME: 1.00 H#: NO SAMPLE REPEATS: 1 DATA CALC: CPM
PRINTER: STD SCR:YES REPLICATES: 1 COUNT BLANK: NO
RS232: OFF RCM:YES MULTIPLIER: 1.000000

ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE:YES

SAM NO	POS	TIME MIN	SCR	32P CPM	32P %ERROR	RCM	ELAPSED TIME
1	1-1	1.00	1.000	336227.44	0.34	0.00	1.73

1/400th x 4 = 1,344,808 x 100 = 134,480,800

Denatured & hyb'd filters 0/n 42°c



To Page No. 73

Witnessed & Understood by me, Date

Invented by W.M. Barnett Date THURS 9/16/94

Recorded by

Project 1713

74 Book No. 18002 TITLE

From Page No. 73

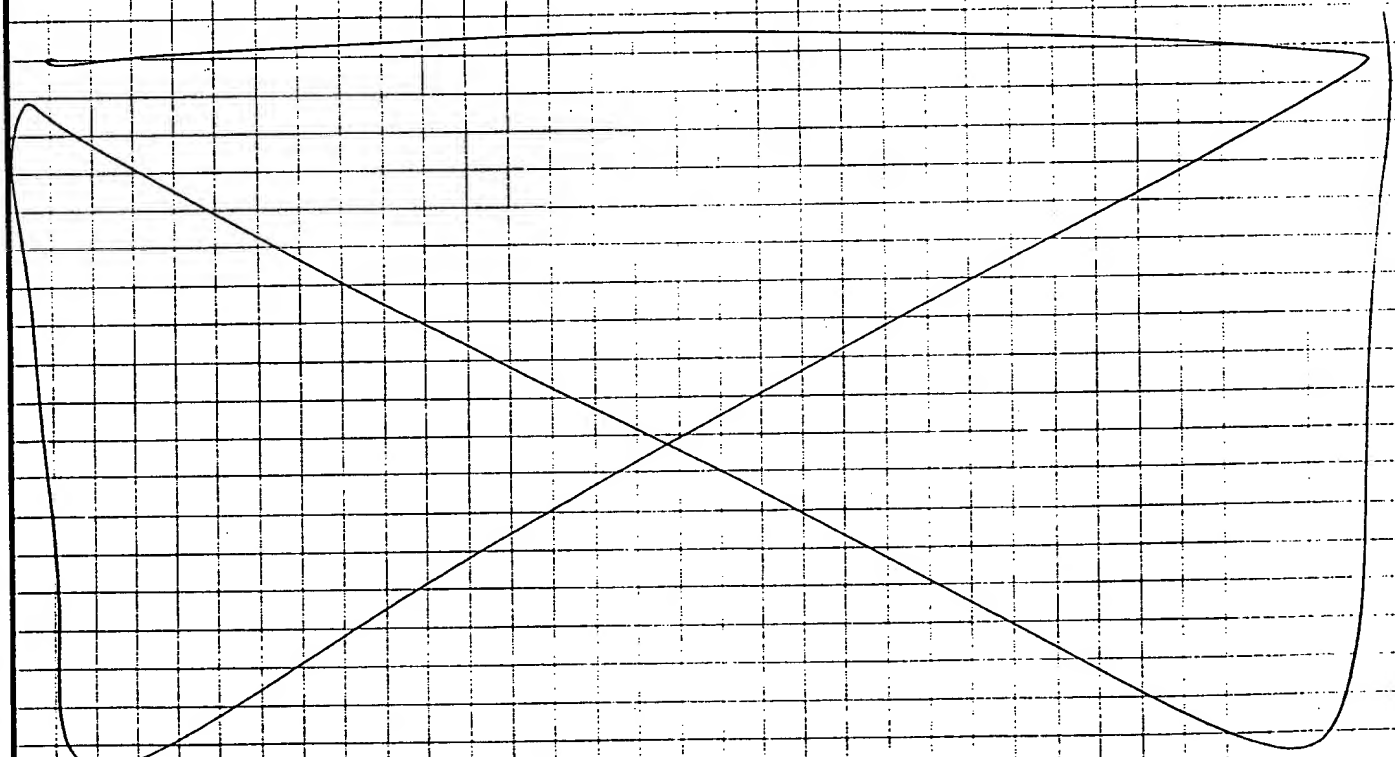
Split plates (Fus 9 & 11) 1:10 each
Also started 100ml spinners for each.

Checked 1L spinners → May start PS04 on Monday.

Washed o/N MnBall 2°'s
2x/2xSSC RT 15'
1x/1xSSC 50°C 30'

Air dried, mounted

A/R'd -70°C until 9/20



To Page No. 75

Witnessed & Understood by me,	Date	Invented by	Date FRI
		Recorded by WM Bonin	9/17/93

TITLE

From Page No. 74

Developed 3 day Rubell 20's
Made Picks → replated 30's O/N.
These do not look very good.

Started 4 x 1L PSO4 for Back FWS 9 & FW

Plus [illegible] to add up for another

[illegible]

To Page

ge No. 75

Witnessed & Understood by me,	Date	Invented by	Date MON
		Recorded by WM Bacon	9/20/93

Project No. 1713

76 Book No. 18002 TITLE

From Page No. 75

Check spinners
Split plates

Did Lifts on O/N MuBall 3°'s
Den, rent, wash, X-linked, Baked
Prehyb'd 20% F 42°C @ 6 hrs

Made transmembrane probe via PCR
purified + counted

USER: 1 ID:32P COMMENTS:32P COUNTING
PRESET TIME: 1.00 H#: NO SAMPLE REPEATS: 1 DATA CALC: CPM
PRINTER: STD SCR:YES REPLICATES: 1 COUNT BLANK: NO
RS232: OFF RCM:YES MULTIPLIER: 1.000000

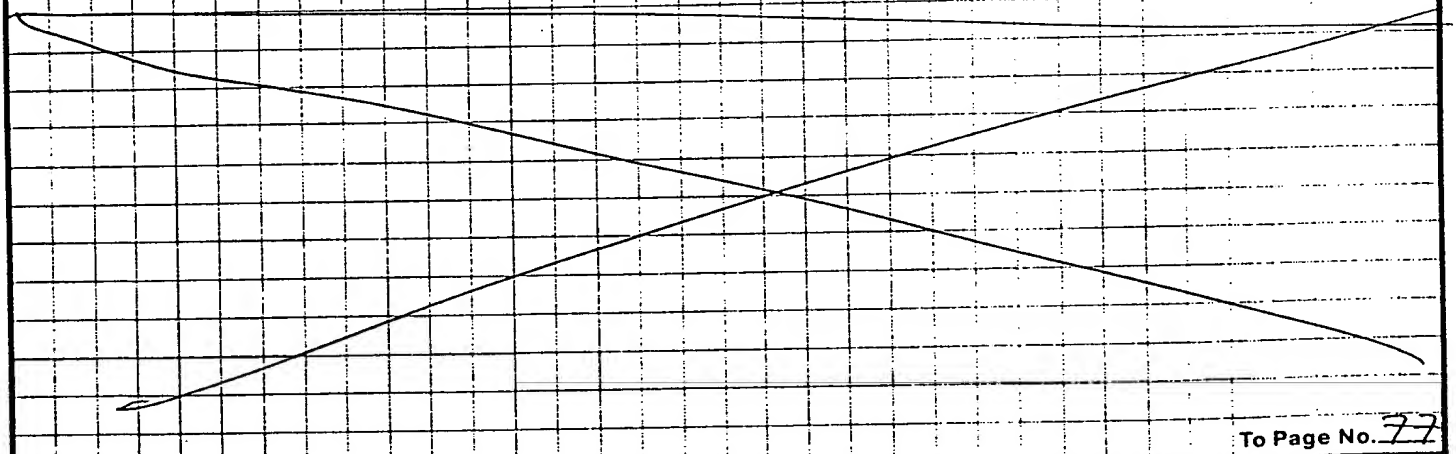
ISOTOPE 1: 32P %ERROR: 0.00 BKG. SUB: 0 HALF LIFE:YES

SAM NO	POS	TIME MIN	SCR	32P CPM	32P %ERROR	RCM	ELAPSED TIME
1	1-1	1.00	1.000	715181.81	0.24	0.00	2.69

0.5% x 2 = 1,430,362 x 100 = 143,036,200

1.4 x 10^8

Denatured + hyb'd O/N 42°C



To Page No. 77

Witnessed & Understood by me,	Date	Invented by Will Bacon	Date THES 9/21/93
		Recorded by	

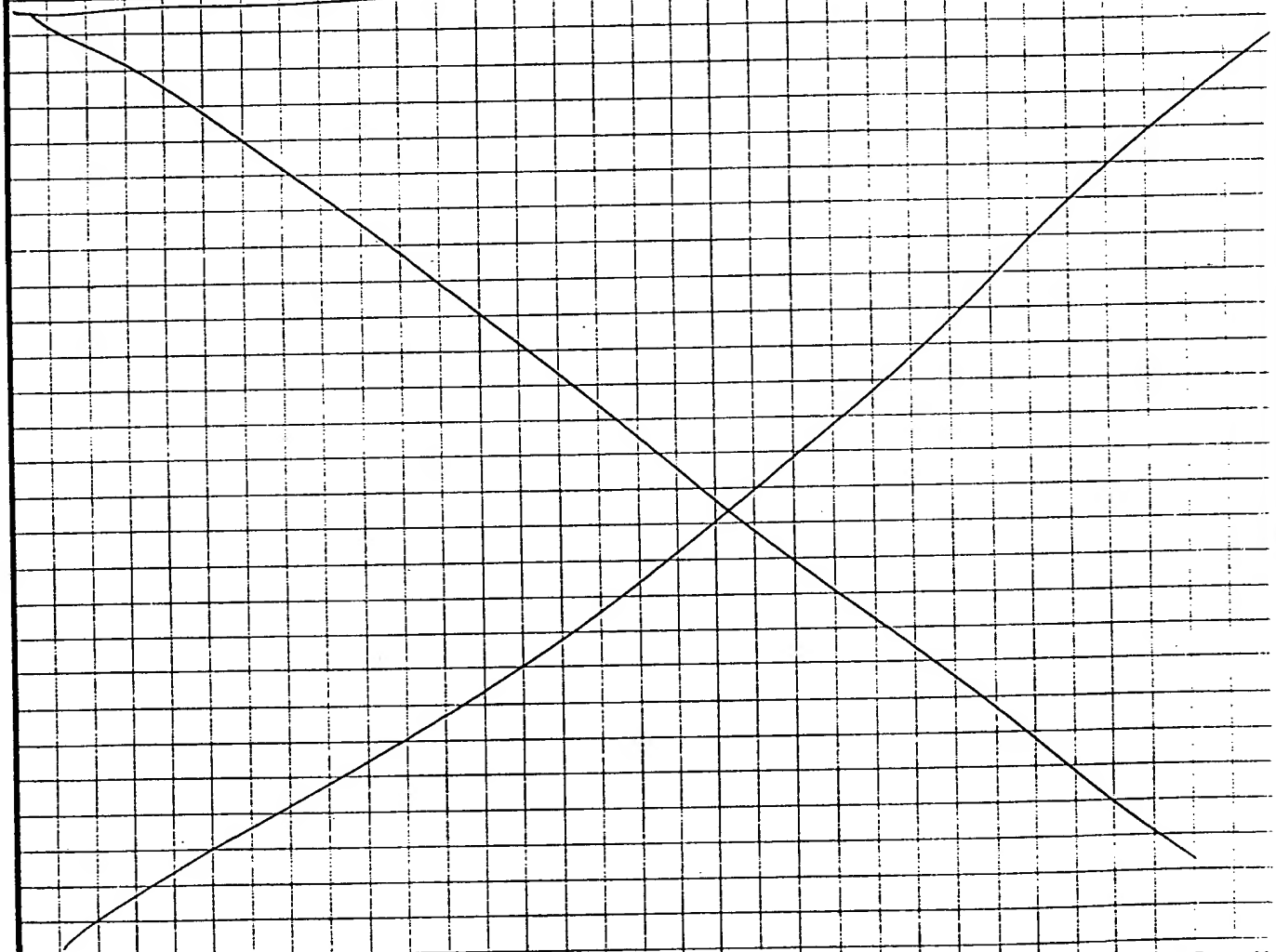
TITLE

From Page No. 76

Split 100ml spinners
Checked PSO4 cultures - going good

Washed O/N filters 2x/2xSSC RT 15'
1x/1xSSC 50°C 30'

Dried, mounted H/R'd -70°C O/N



To Page No

Page No. 77

Witnessed & Understood by me,

Date

Invented by Will Bacon

Recorded by

Date WED 9/22/93

78 Project No. 1713 Book No. 18002 TITLE _____

From Page No. 77

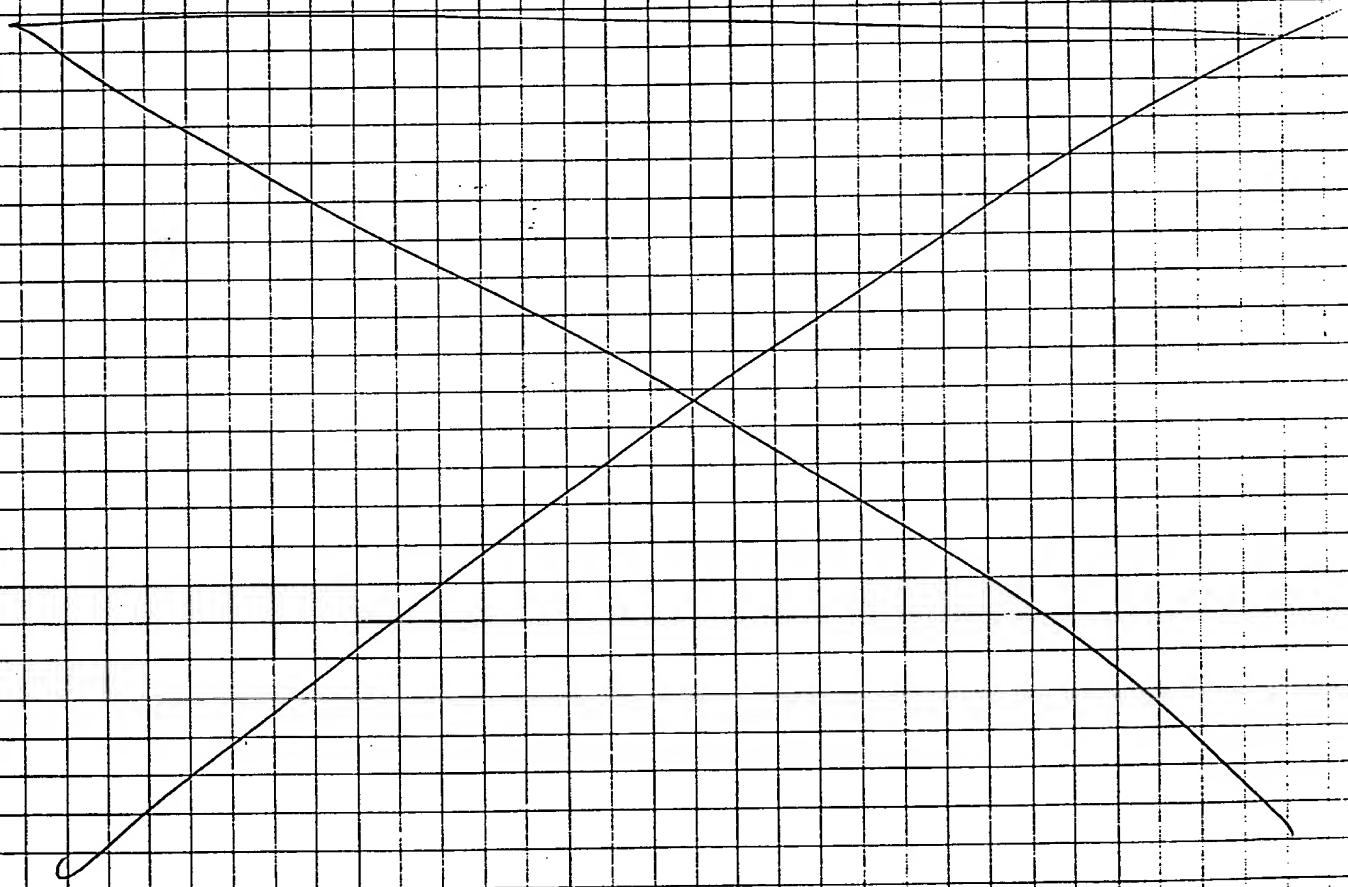
Developed O/N MuBall A/R's

There ~~are~~ is perhaps 3 positives

Picked ~~Picked~~ & eluted in PSB. Stored 4°C O/N

FT

Checked spinness



To Page No. 79

Witnessed & Understood by me, | Date | Invented by | Recorded by WM Barron | Date THURS 9/23/93

TITLE

From Page No. 78

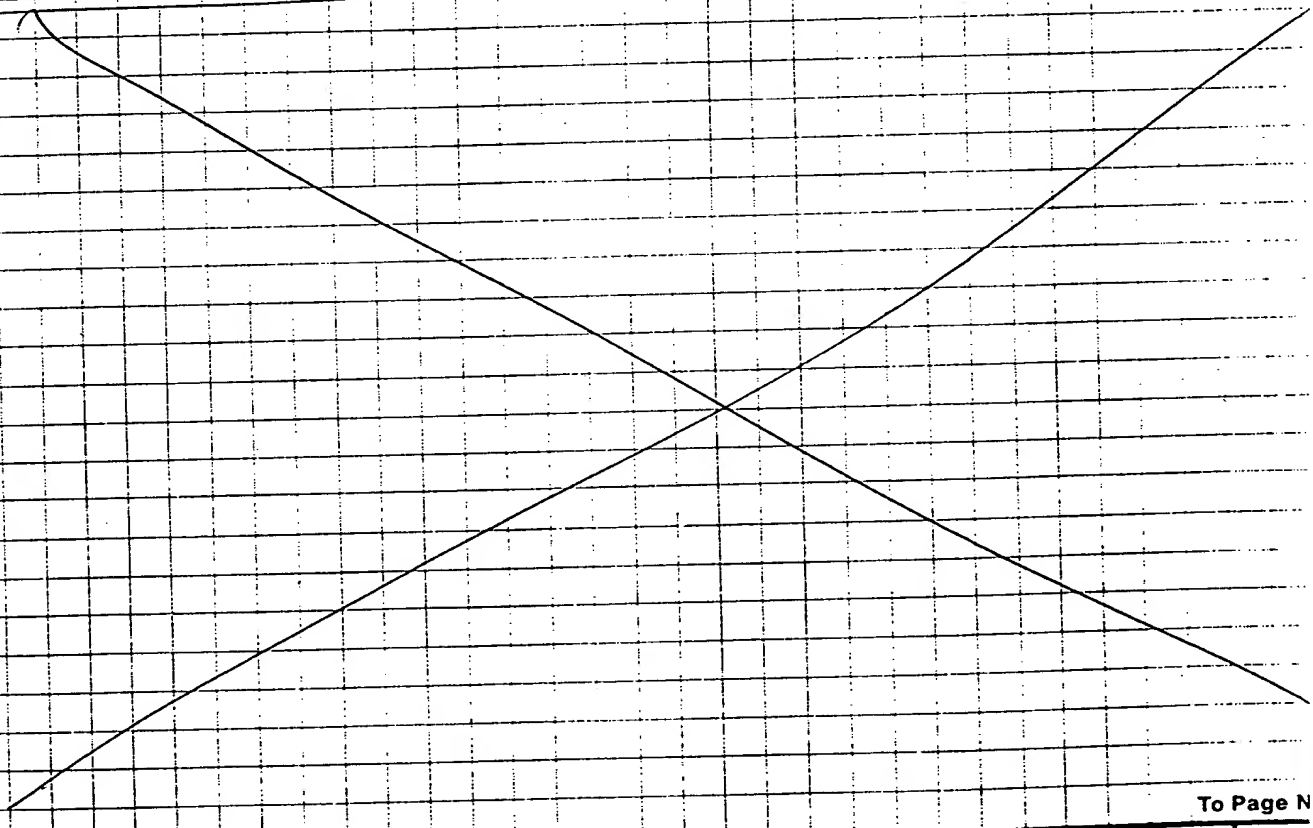
Started O/N MuBall MIDI's w/ 10µl & 100µl ~~tips~~ pick
in Sorvall NZ47.OT w/ C600 as Host.

Inc 37°C O/N.

Checked spinners

Spit plates

Started new 100ml spinners on Fxs 9 & 11



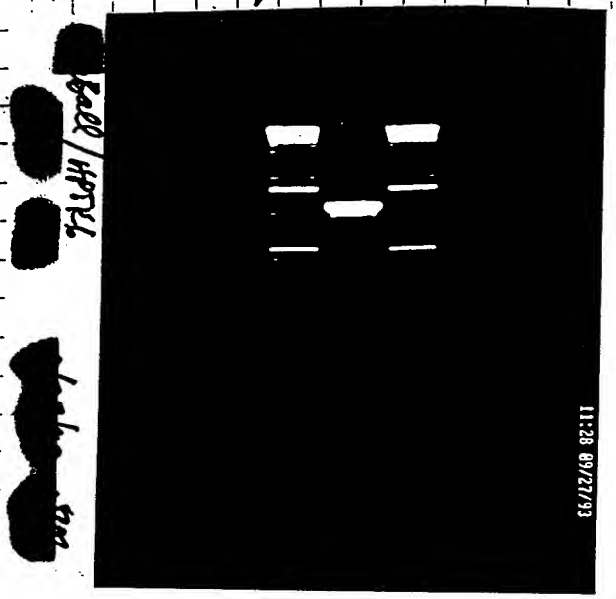
To Page N

Witnessed & Understood by me,	Date	Invented by WM Bacon	Date FRI 9/24/93
		Recorded by	

80 Project No. 1713 Book No. 18002 TITLE

From Page No. 79

Ran a GT10 PCR on MuBall positive to size insert
Ran on a gel



Insert is ~ 1.2 Kb

Split 100ml spinners

Split plates

Checked PS04 cultures

To Page No. 87

Witnessed & Understood by me,	Date	Invented by / Recorded by Will Bacon	Date MON 9/27/93

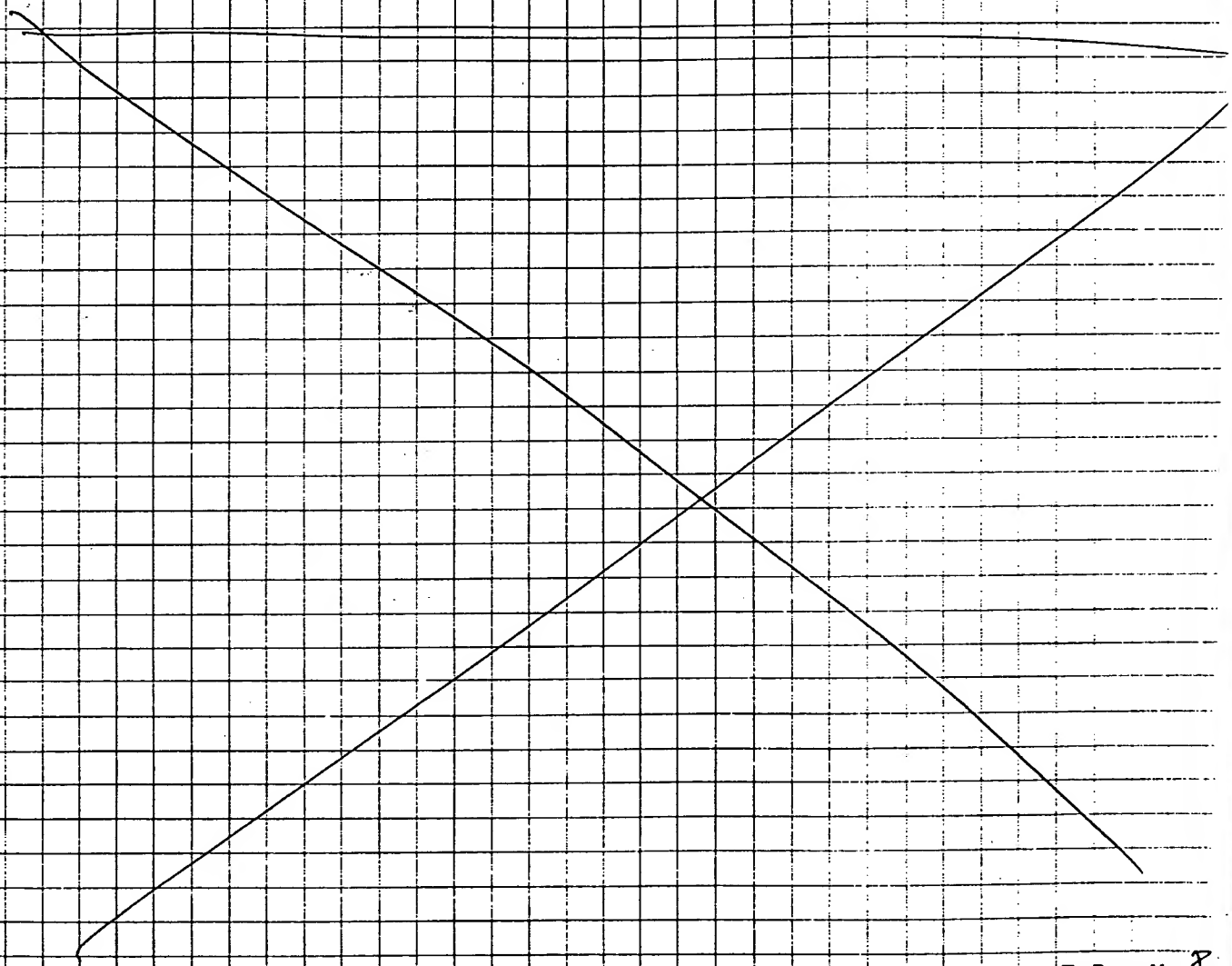
From Page No. 80

Checked spinners + plates

PCR sequenced (Enrol) MuBall TKG

Ran on a wedge gel

A/R'd 2 hrs then o/N.



No. 81

To Page No. 8

Witnessed & Understood by me,	Date	Invented by	Date TUES
		Recorded by Will Barrett	9/28/93

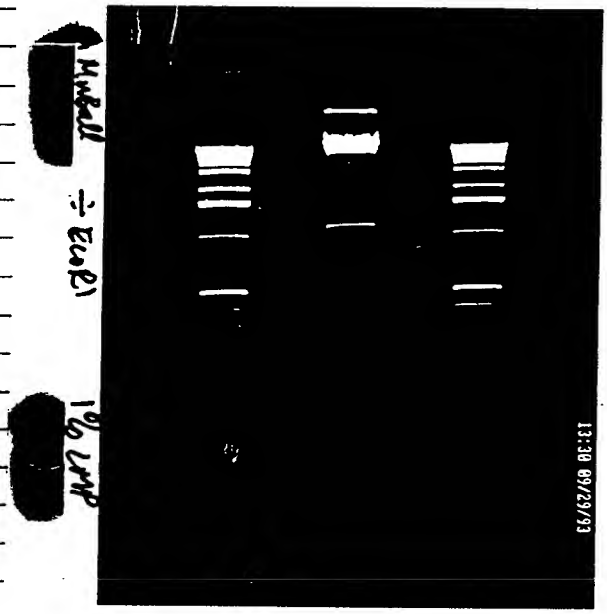
From Page No 81

Developed O/N Seq A/R → could not read

I will subclone into Bluescript

Need to cut 1st w/ EcoRI

RD'd 10µl 1 DNA → ran on 1% LMP



Cut out indicated band

Magic PCR prep

Ligated to SKΘ O/N 12.5°C.

Checked all spinnes & plates.

To Page No. 83

Witnessed & Understood by me,	Date	Invented by	Date WED
		Recorded by WM Bacon	9/29/93

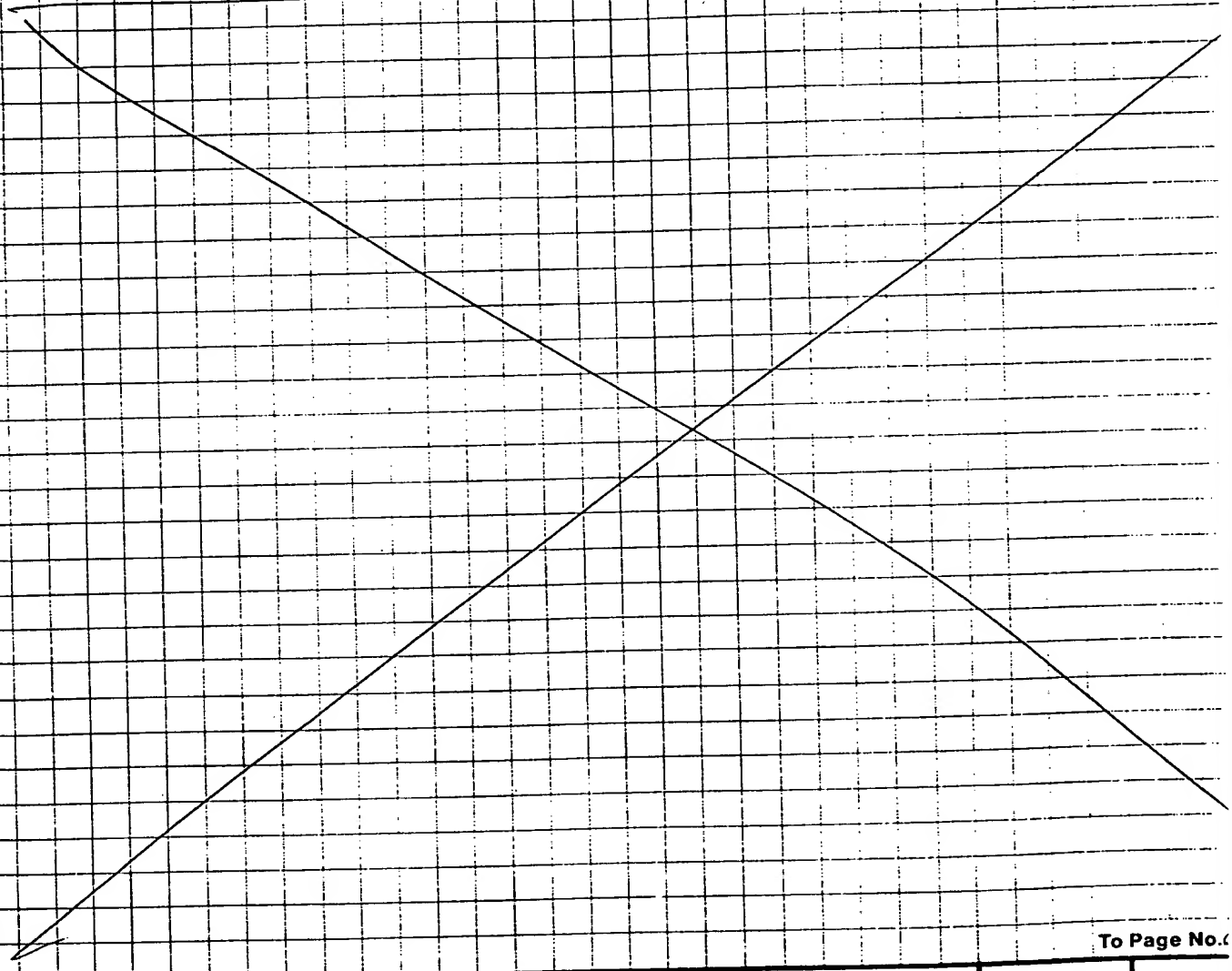
TITLE

From Page No. 82

Transformed O/N SKG/MuTK6
Inc. 37°C O/N

Checked all spinners & plates
Split all.

Checked PS04 cultures → cont Incs.



To Page No.

No. 83

Witnessed & Understood by me,	Date	Invented by	Date THURS
		Recorded by	9/30/93

Project No. 1713

84 Book No. 18002 TITLE

From Page No. 83

Checked O/N MnTK6/SK8 trans plates

Started 20x 5mil O/N MP's + masters

checked spinners & plates

Cont Zinc on PS04's

To Page No. 85

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		W. M. Bacon	FRI 10/1/93

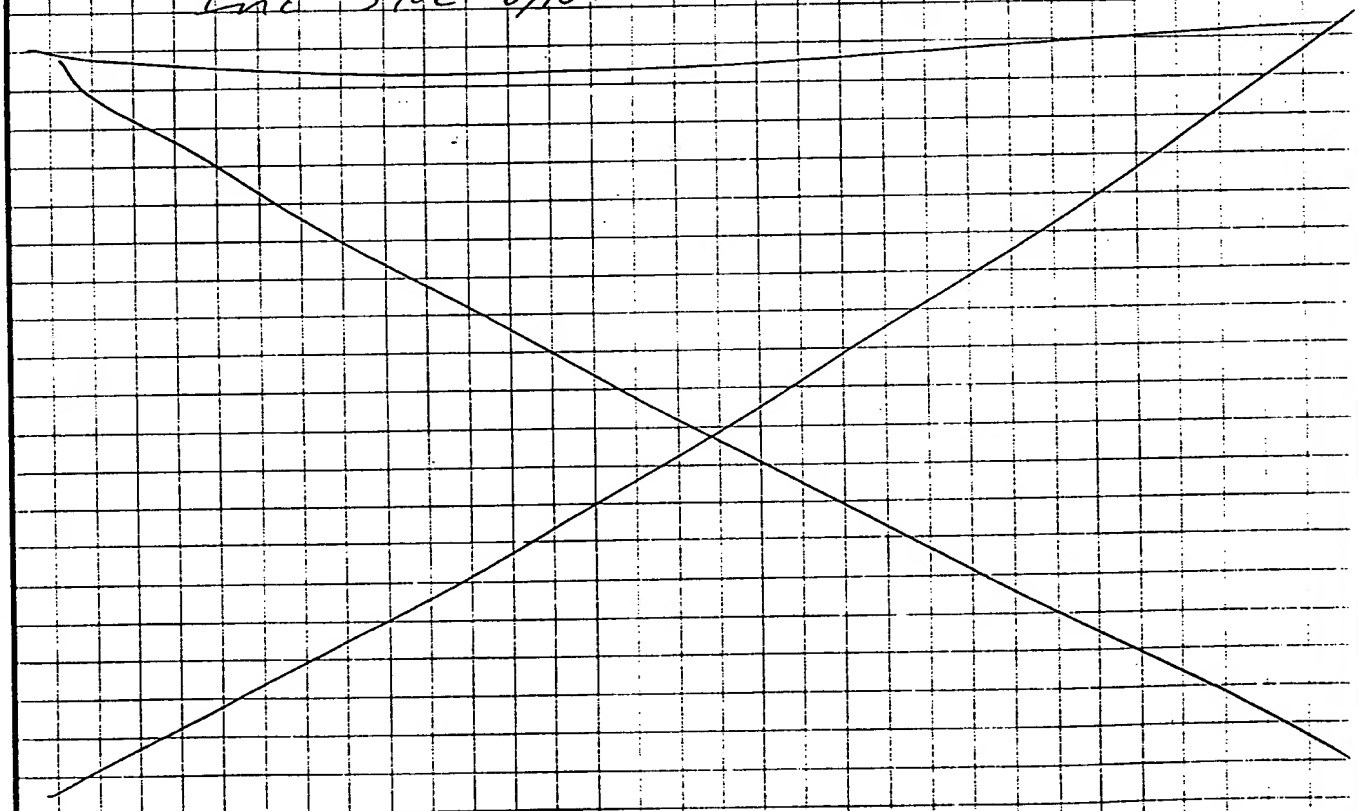
From Page No. 84

Harvested all 8 L of PSO4 → filtered
Added protease inhibitors (Aprotinin, PMSF, Leupeptin, Pepstatin

Run Prot A column on (1st ~~[illegible]~~ ~ 3L (as much as I could
~~[illegible]~~ Load in ~ 8 hrs) (stored remainder 4°C o/N)
washed, eluted, desalted, stored 4°C

Split all spinners + plates

To make sure the MuTK6/3K0 were ~~[illegible]~~ not
blue colonies (color rxn was weak at transformation
I restreaked all 20 onto LB cart + XGal/IPTG
Incu 37°C o/N.



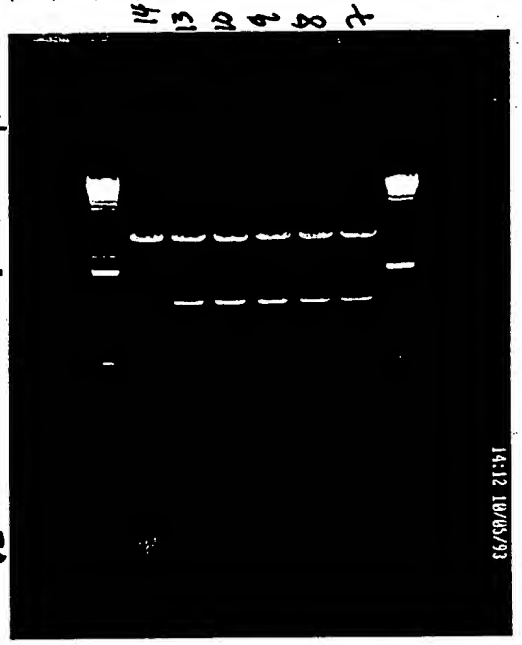
To Page No. 85

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		WM Barnett	MON 10/4/93

86 Project 1713 Book No. 18002 TITLE

From Page No. 85

Checked restreaks of M₁₄TK6/SK- to see if blue or white.
Of 20 only 6 were white!
Did Magic MP's on These 6
Cut w/ EcoRI + ran gel



Denatured 10µl #7 for sequencing
Neutralized + EtOH ppt'd O/N.

Continued running Prot A column on PS04 TK6/Ig6
washed, eluted, desalted
Stored 4°C
(combined w/ run from 10/4)

Checked all spinners + plates

To Page No. 87

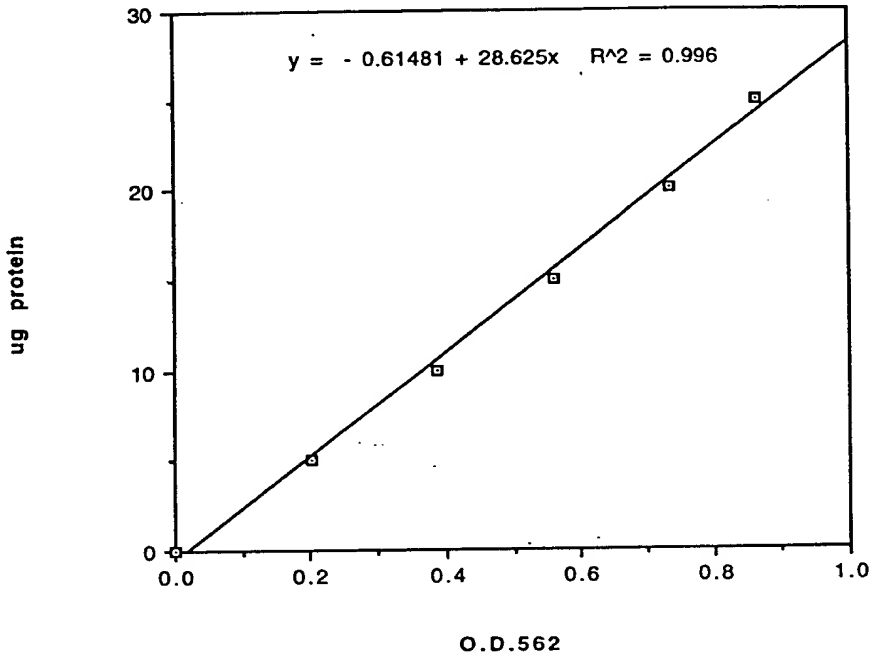
Witnessed & Understood by me,	Date	Invented by / Recorded by WM Bacon	Date TUES 10/5/93

TITLE

From Page No. 86

Ran BCA on 1st 2 Prot A runs of TF6/Ig6

Data from "Untitled Data #1"



S1 = 0.203
S2 = 0.387
S3 = 0.560
S4 = 0.735
S5 = 0.864
Fus = 0.449

x = 0.449

y = 28.625(.449) - 0.61481

y = 12.2µg

in 100µl

122ng/µl

To Page No. 8

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date WED 10/6/93
		W.M. Barron	

Project No. 1713
88 Book No. 18002 TITLE

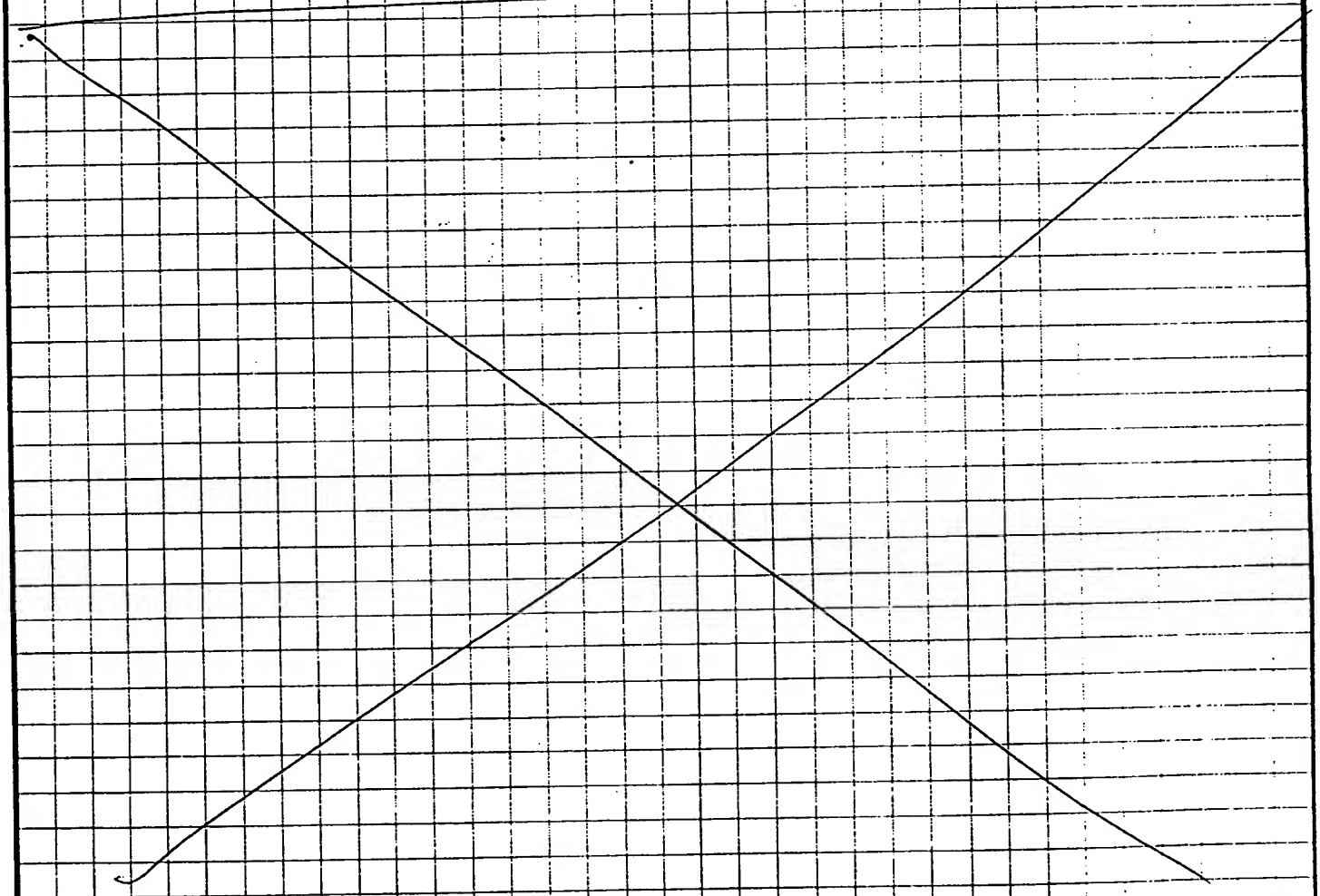
From Page No. 87

Aliquoted protein & stored -70°C

Ran next ProtA

washed, eluted, desalted → stored 4°C

Checked spinners & plates



To Page No. 89

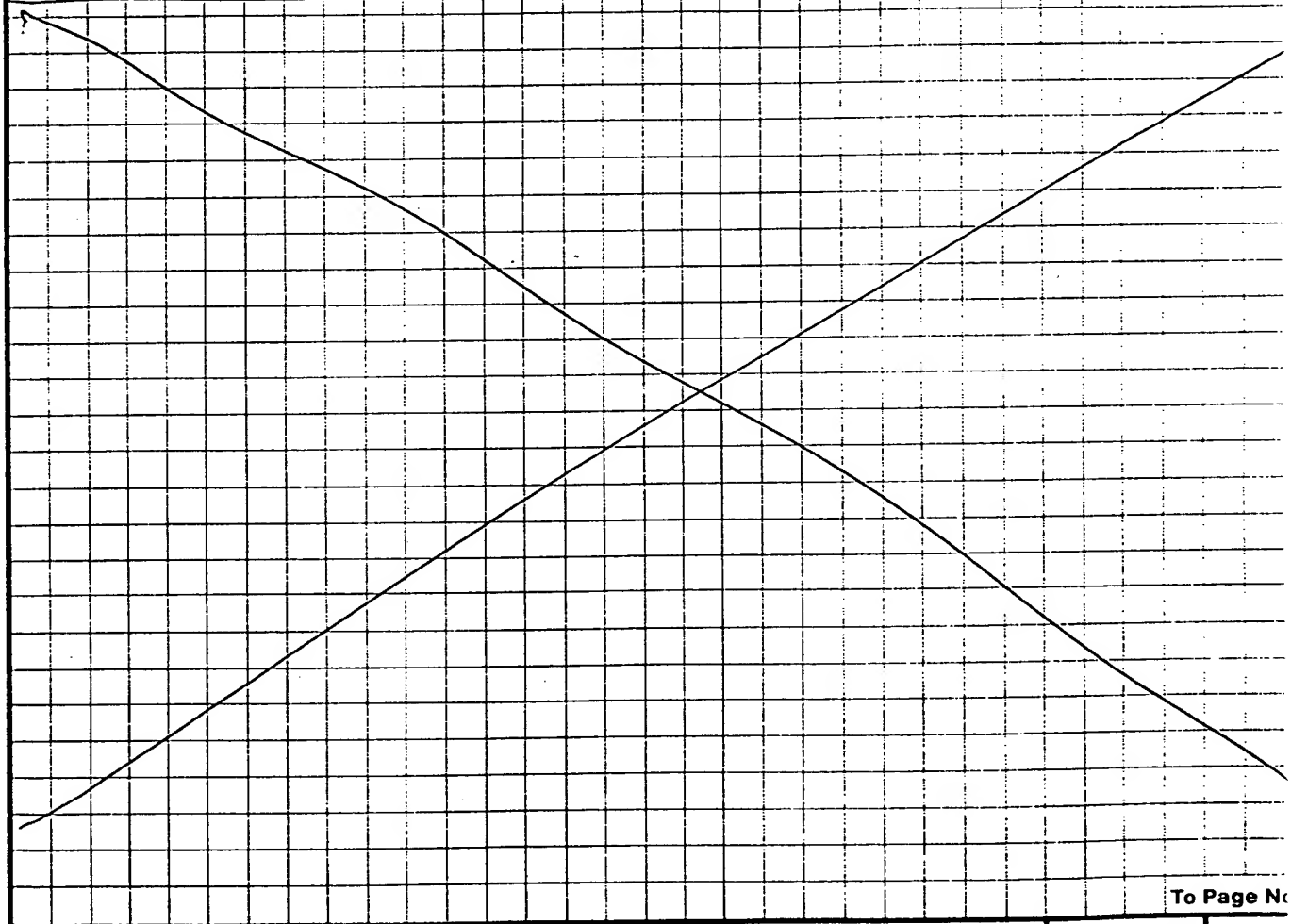
Witnessed & Understood by me,	Date	Invented by	Date WED
		Recorded by WM Bacon	10/6/93

TITLE

From Page No. 88

Ran next prot A column on TK6/Tg-6
Washed, eluted, desalted
Stored 4°C O/N.

Did 5eq rxns on Mu TK6/sK- #7
Ran on 2 seq gels → Dried, H/R'd RT O/N



ge No. 89

To Page No

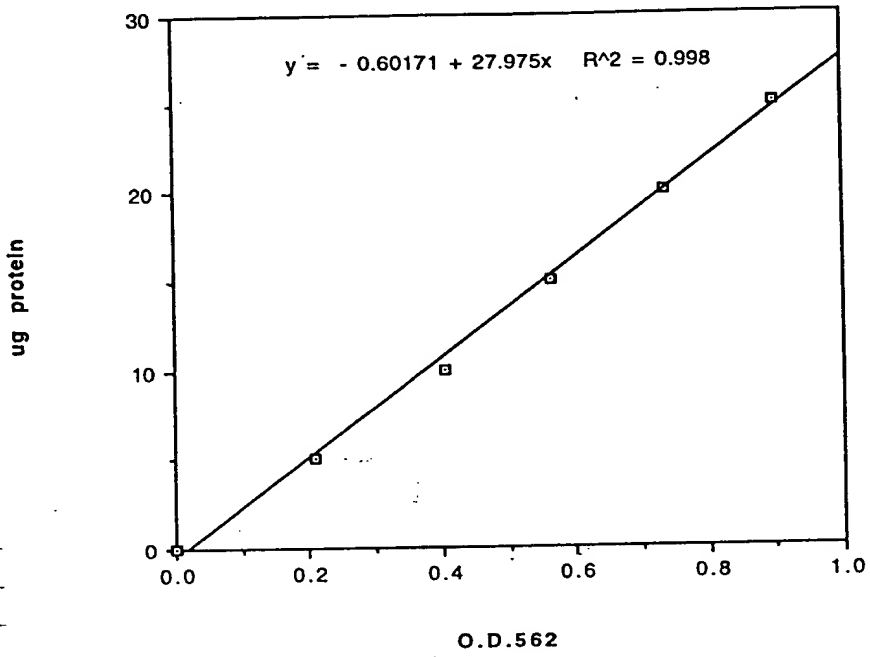
Witnessed & Understood by me,	Date	Invented by	Date THURS
		Recorded by [signature]	10/7/93

Project No. 1713

90 Book No. 18002 TITLE

From Page No. 89 BCA's done on TK6/Ig6 from pgs 88-89

Data from "Untitled Data #1"



S1 = 0.208
S2 = 0.403
S3 = 0.565
S4 = 0.734
S5 = 0.900

p.88 sample = 0.381
p.89 sample = 0.393

A) $y = 27.975(0.381) - 0.60171$

$y = 10.06\ \mu g / 100\ \mu l$

$= 101\ ng/\mu l$

B) $y = 27.975(0.393) - 0.60171$

$y = 10.4\ \mu g / 100\ \mu l$

$= 104\ ng/\mu l$

D

Witnessed & Understood by me,	Date	Invented by Wm Bacon	Date Fri 10/8/93
		Recorded by	

TITLE

From Page No. 10

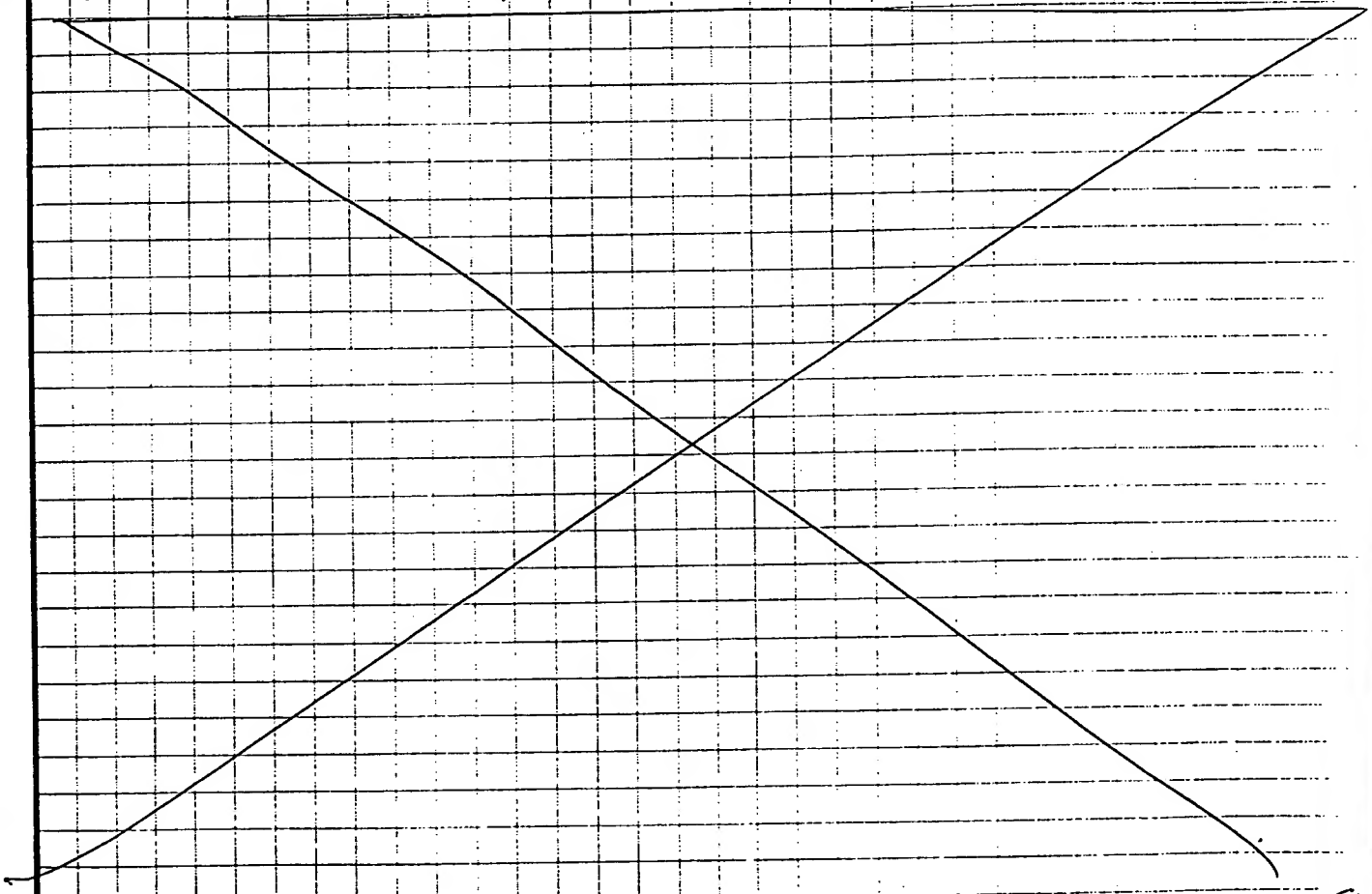
Aliquoted samples & stored -70°C

Developed Seq A/R's & read

MuTK6 is not Mouse HPTK6. Other Mouse junk.

Decided to scrap this project.

Split spinners & plates



To Page No. 9

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date
		Will Bacon	FRI 10/8/93

Project No. 1713

92 Book No. 18002 TITLE

From Page No. 91

Ran SDS-PAGE gel on all new currently purified TK6/IgG samples (8 total)

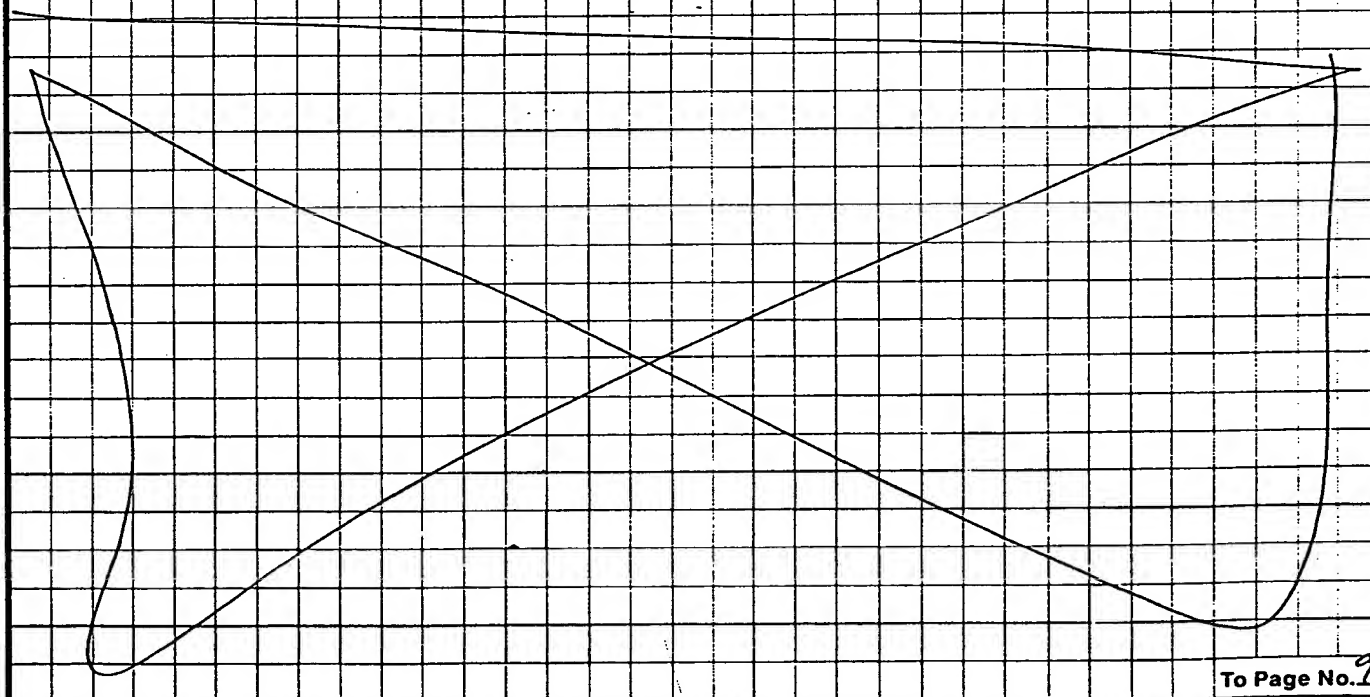
1 (which is 1 & 2 combined) 5µg = 41µl

2 (which is 3RD) 5µg = 50µl

3 (which is 4TH) 5µg = 50µl

Fixed, stained w/ Coomassie Blue

Destained O/N.



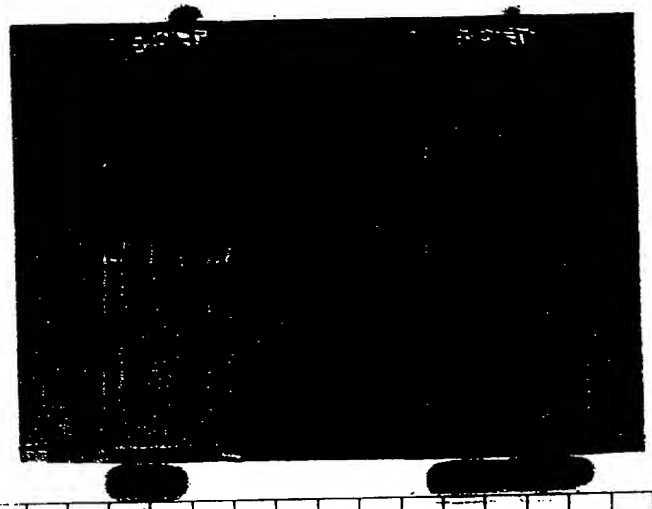
To Page No. 93

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by Jill Barron	SAT 10/9/93

TITLE

From Page No. 92

Photographed SDS-PAGE of TEC/Tg6 samples



Samples intact.

Transferred to
B. Fendly &
G. Bennett
for Antibody productio

Checked spinners & plates.

To Page N

Witnessed & Understood by me,	Date	Invented by WM Bacon Recorded by	Date MON 10/11/93

Project No. 1713
Book No. 18002 TITLE

Page No. 93

plot spinners & plates

2 am next Prot A column washed, eluted, desalted
Stored 4°C

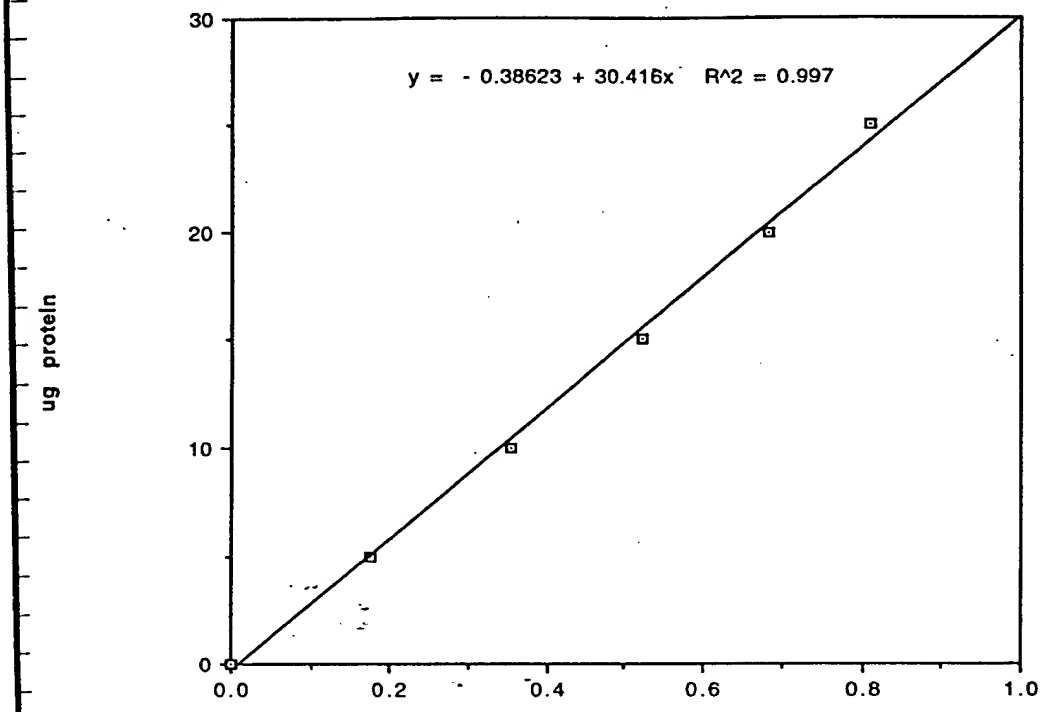
To Page No. 9

Witnessed & Understood by me, | Date | Invented by | Recorded by | Date 10/12/93

Projec
Boo

TITLE

From Page No. 94 Did BCA's on next batch of TK6/Ts G



S1 = 0.176
S2 = 0.352
S3 = 0.522
S4 = 0.682
S5 = 0.810
FUS = 0.374

FUSRUN = 0.374

[redacted]

y = 30.416(0.374) - 0.38

y = 11 µg Protein (

≐ 110 ng/µl

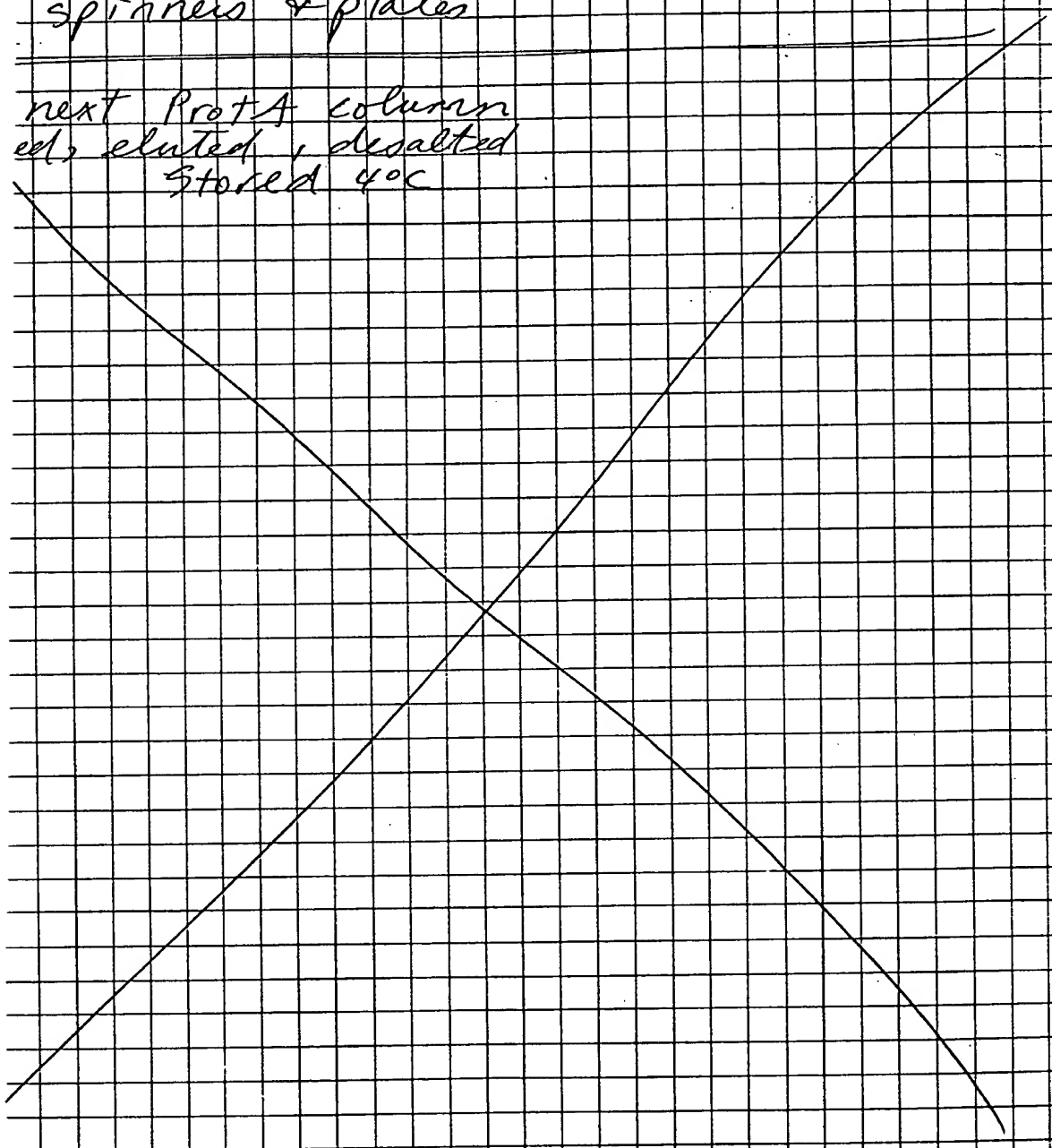
Witnessed & Understood by me, | Date | Invented by Will Bacon | Recorded by | Date [redacted]

Project No. 13
Book No. 18002 TITLE

3

spinners & plates

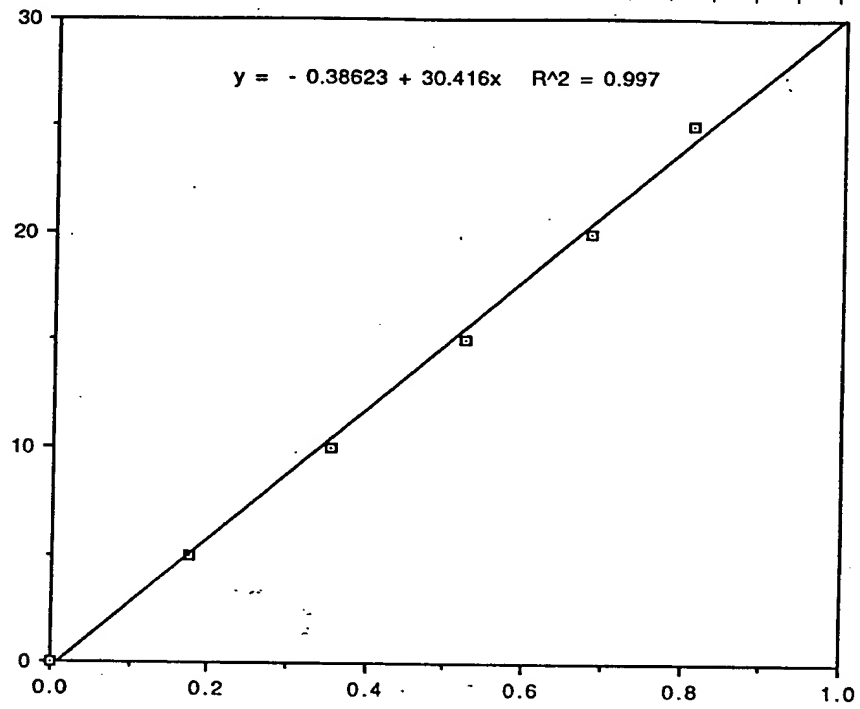
next Prot A column
eel, eluted, desalted
Stored 4°C



To Page No. 95

Understood by me,	Date	Invented by	Date
		Recorded by WMBacon	10/12/93

n Page No. 94 Did BCA's on next bath of TK6/Ig G



S1 = 0.176
S2 = 0.352
S3 = 0.522
S4 = 0.682
S5 = 0.810
FUS = 0.374

FUSRUN = 0.374

[redacted]

$y = 30.416(0.374) - 0.38623$

$y = 11 \mu g$ Protein (in 100 µl)

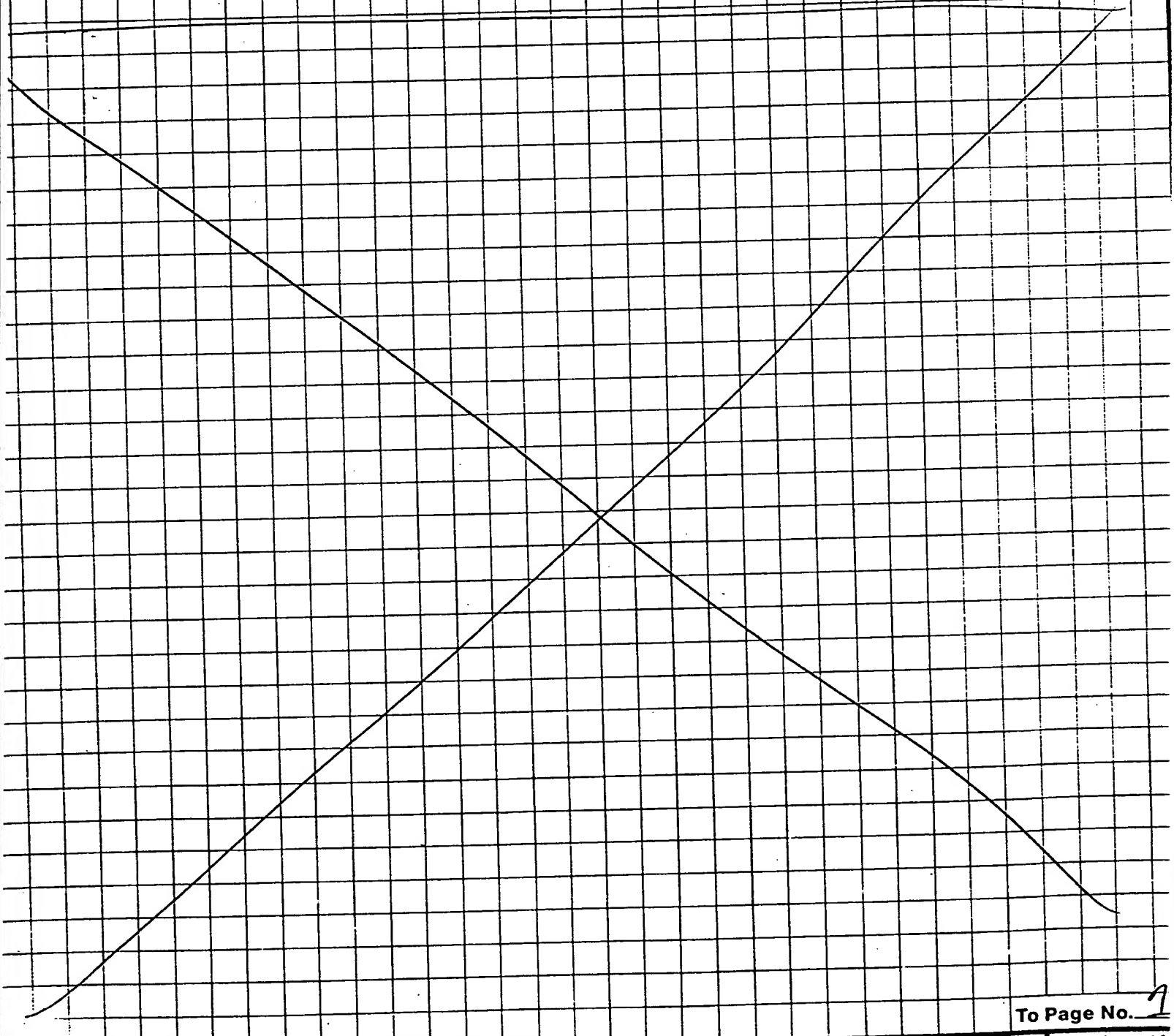
$\hat{=} \frac{110\ ng}{\mu l}$

Witnessed & Understood by me,	Date	Invented by Will Bacon	Date [redacted]	THUI 10/14,
		Recorded by		

Page No. 95

Digested protein + stored -70°C

checked spinners + plate



To Page No. 1

BOOK

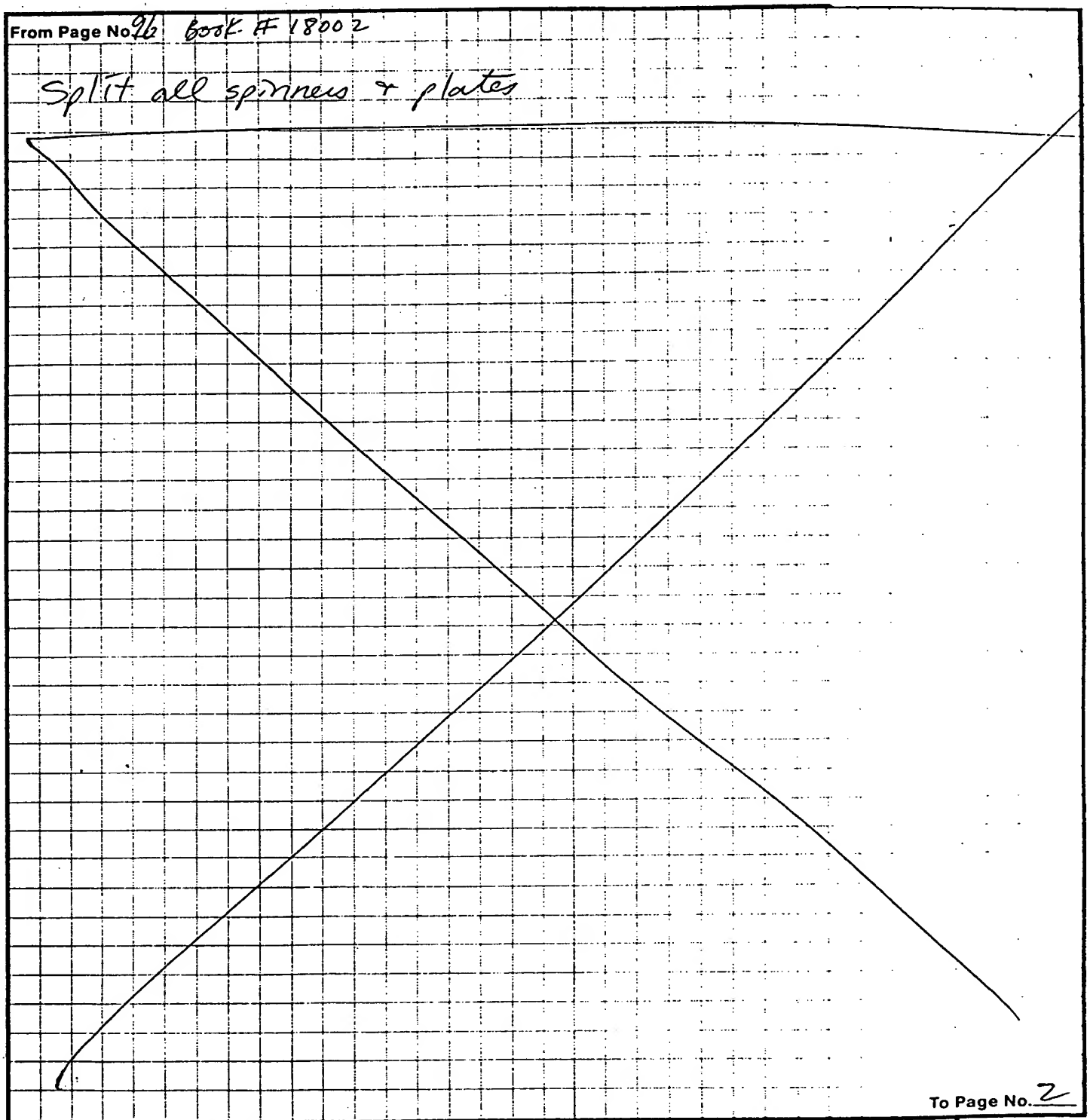
TITLE

Proje lo. 1713

Book No. 199

From Page No. 96 book # 18002

Split all spinners & plates



To Page No. 2

Witnessed & Understood by me,	Date	Invented by	Date FRI
		Recorded by Will Bowen	10/15/93

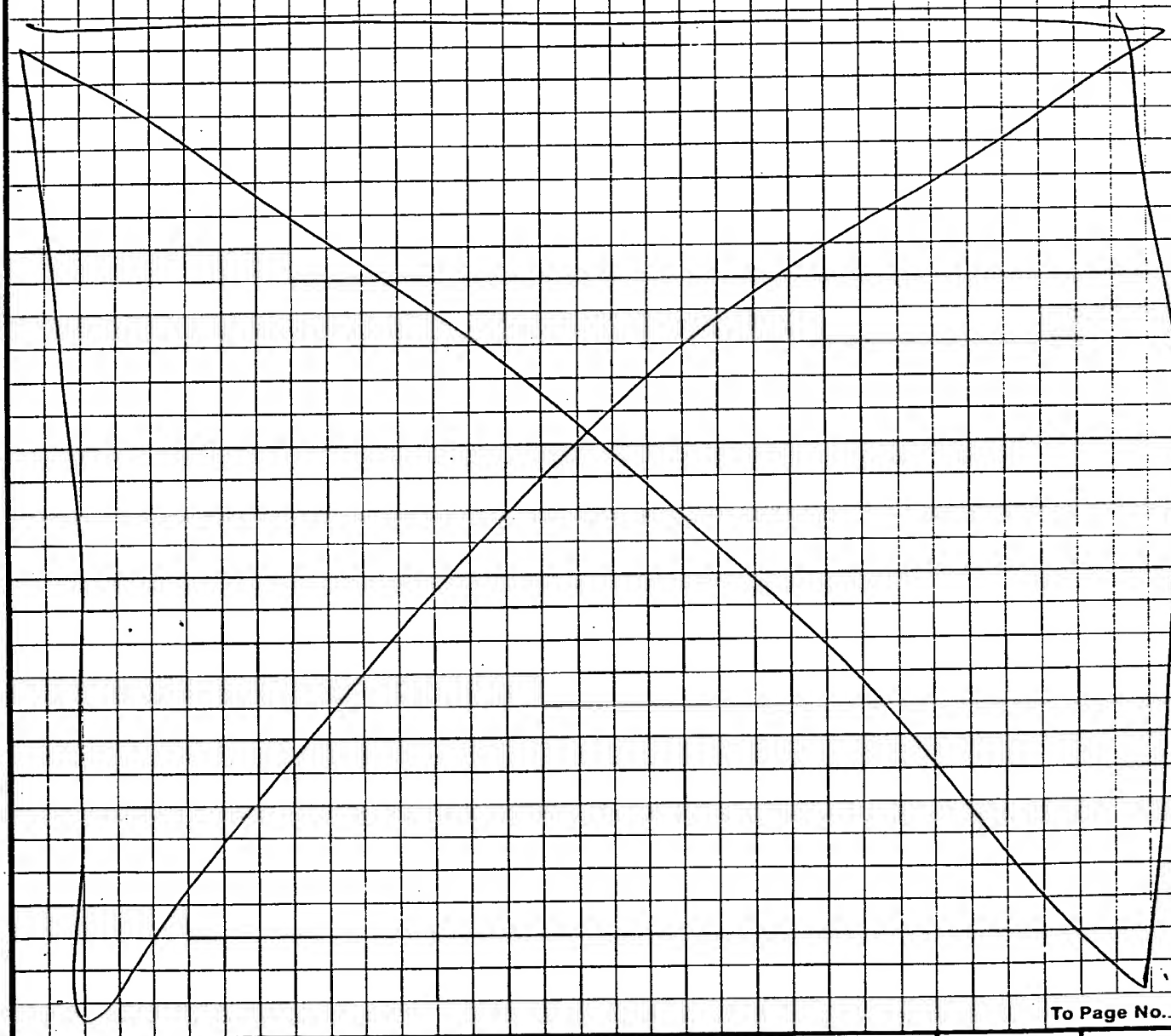
Project No. 1713

2 Book No. 19952 TITLE

From Page No. 1

Split spinners & plates

(CHO / TK6 Jg6 → Fus 11 & 9)



To Page No. 3

Witnessed & Understood by me,	Date	Invented by WM Bacon	Date MON 10/18/93
		Recorded by	

TITLE

From Page No. 2

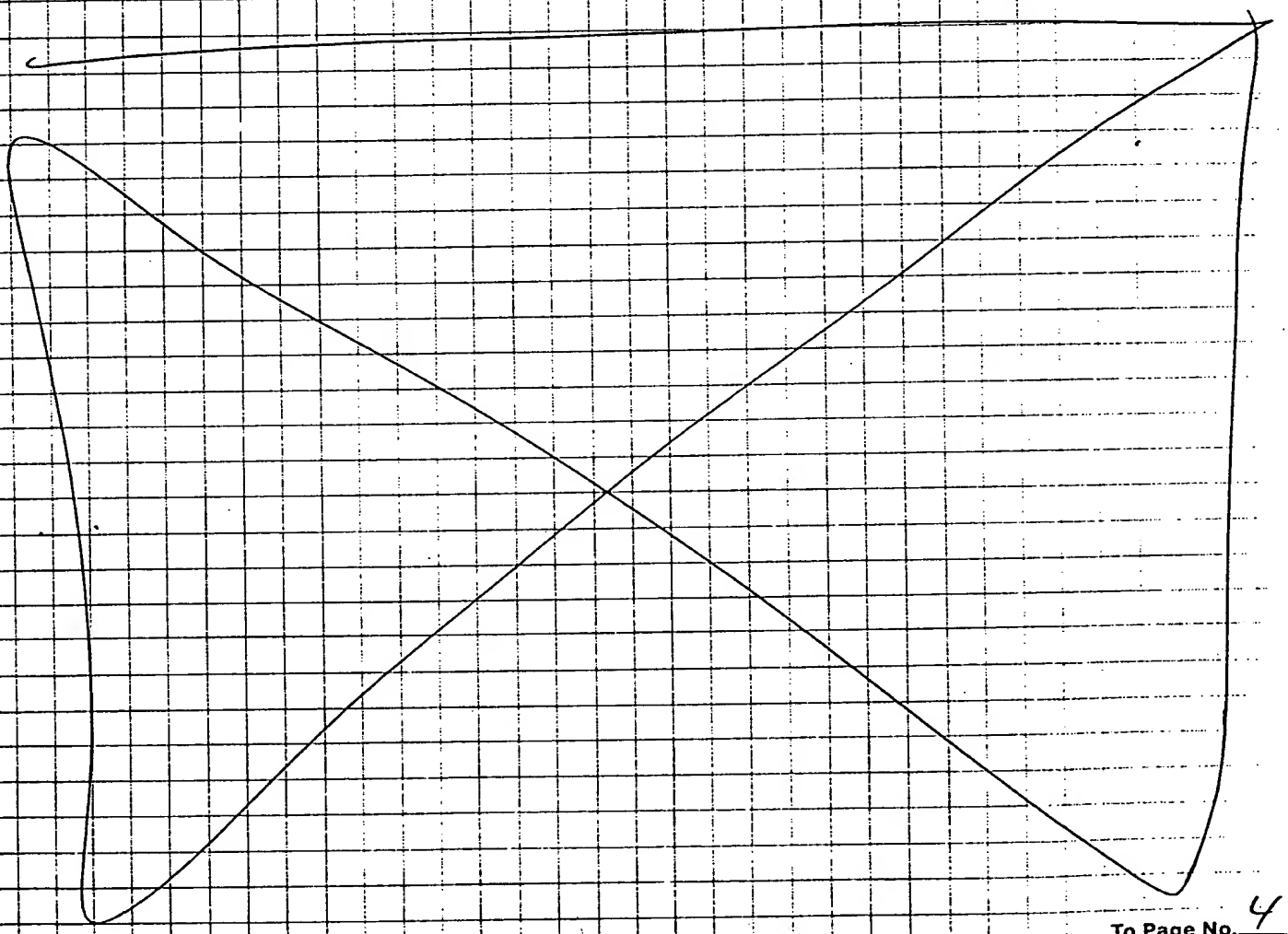
Checked spinners & plates

Got a clone 4 mouse brain library from M. Mark

May try to clone MuTK6 out of it.

Started O/N C600 HFC @ 37°C in N2XDT +/- Maltose Mg2+

Inc 37°C



To Page No. 4

Witnessed & Understood by me,	Date	Invented by	Date
		Recorded by Will Bacon	TUES 10/19/93

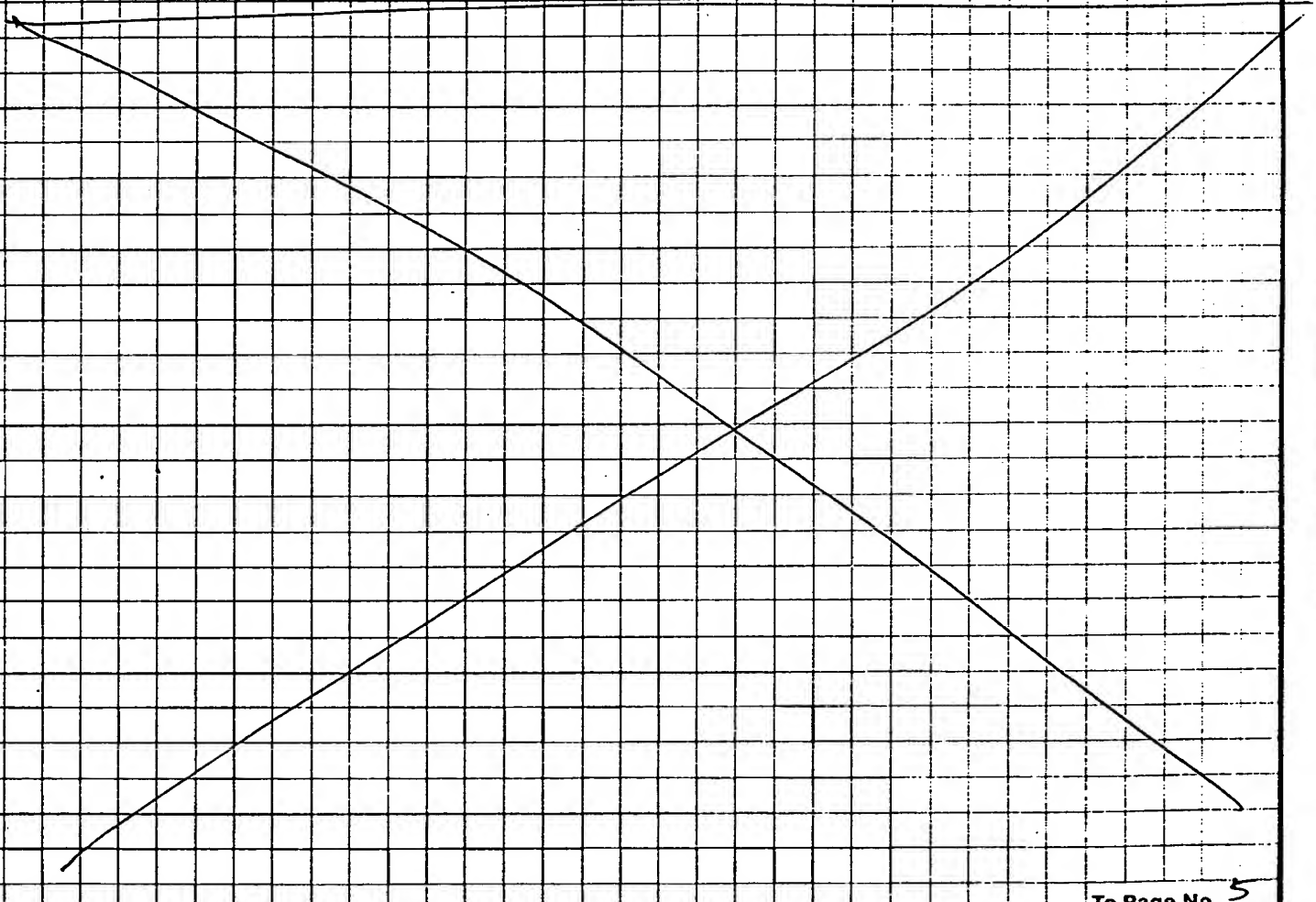
4

Project No. 1713
Book No. 19952 TITLE

From Page No. 3

Plated out Mu Brain Lib O/N
~2×10⁶ pfu (used MM's titer)
Inc 37°C O/N.

checked spinners & plates



To Page No. 5

Witnessed & Understood by me,	Date	Invented by / Recorded by	Date RECD 10/20/93
		Wm Barron	

TITLE

From Page No. 4

Did double litts on MuBrain Library

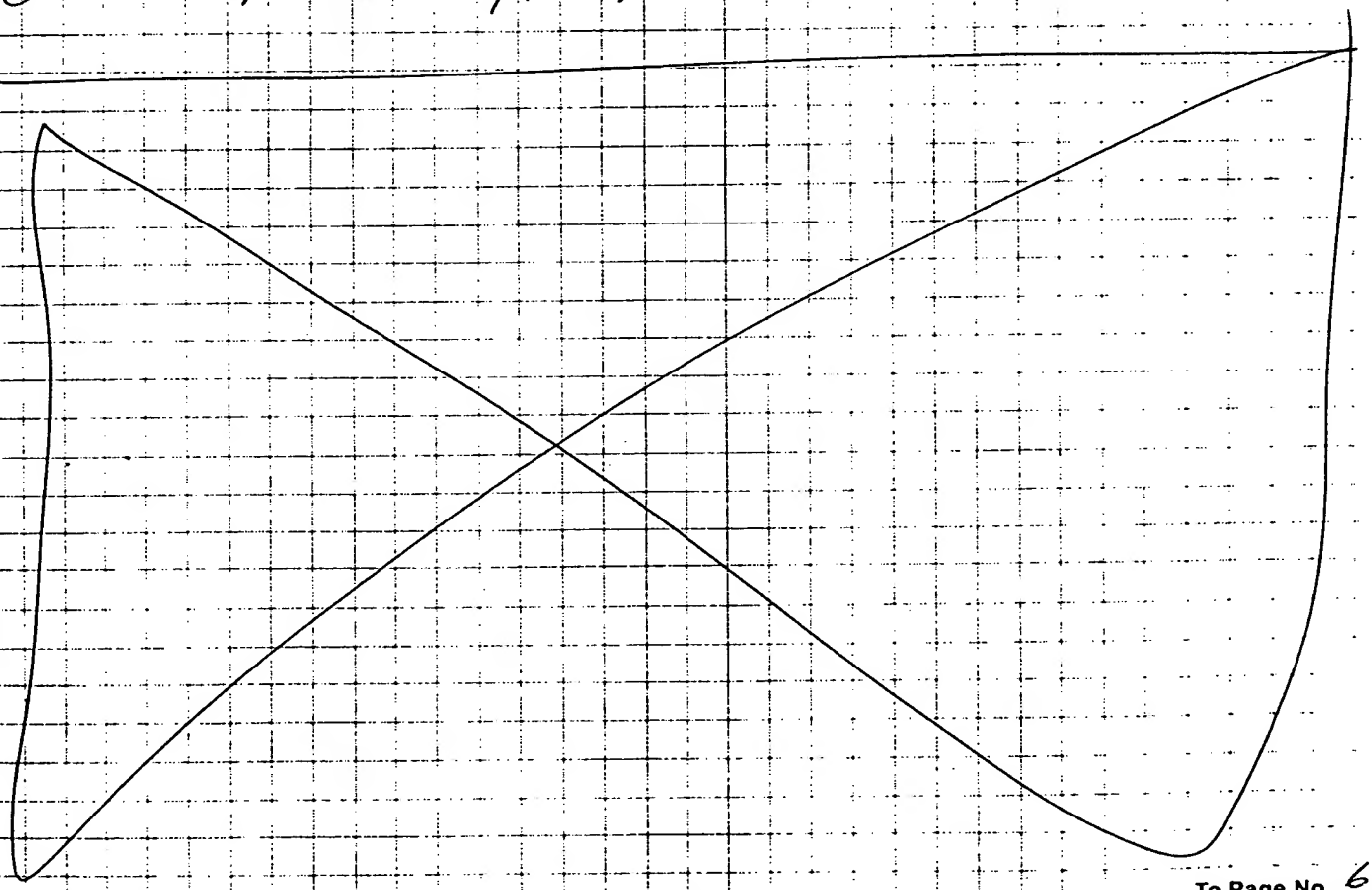
Denatured, neutralized, washed

UV x-linked

Baked

Stored RT

Checked Spinner & plate,



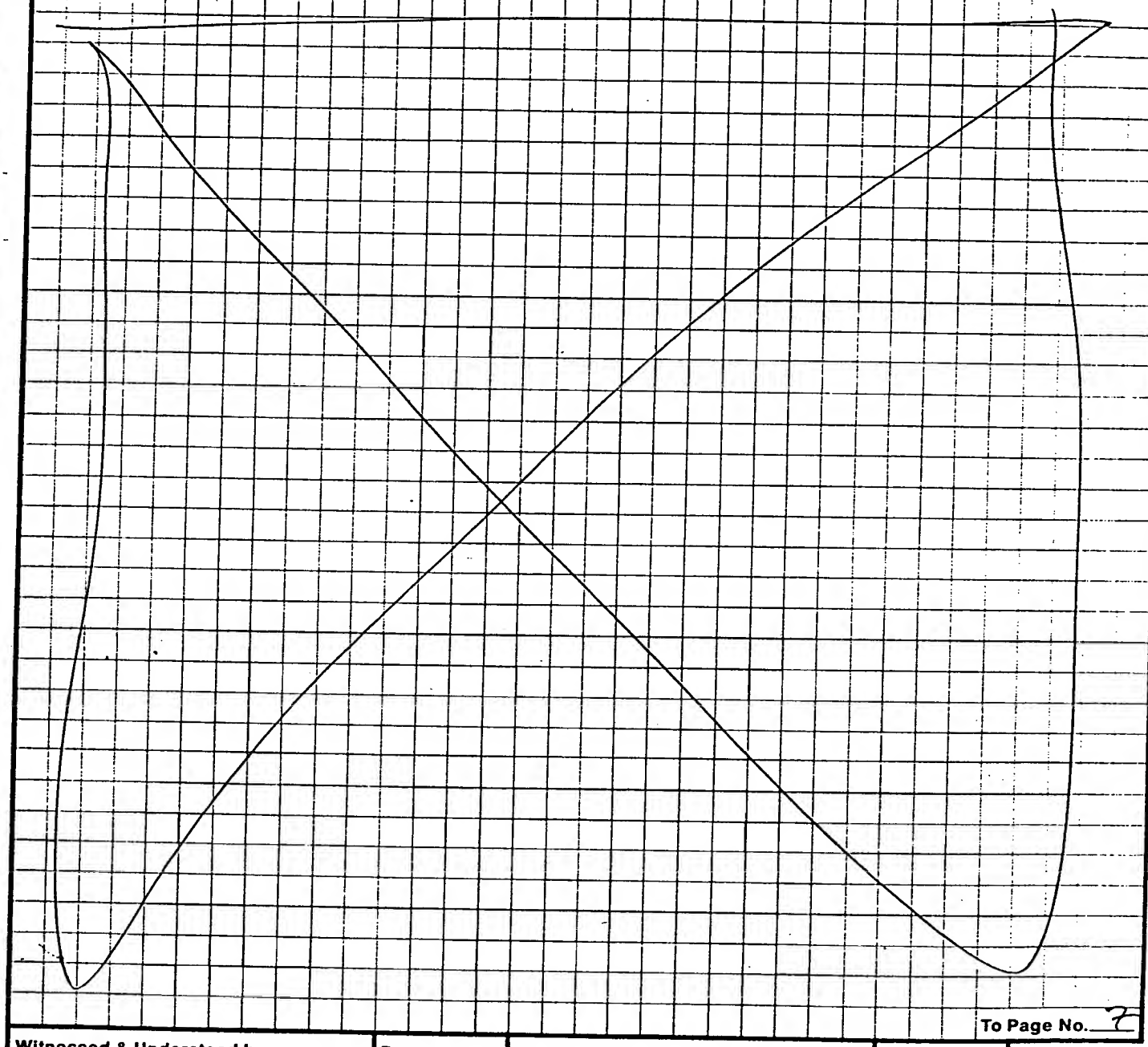
To Page No. 6

Witnessed & Understood by me,	Date	Invented by	Date THURS 10/21/93
		Recorded by Will Bacon	

Project No. 1713
Book No. 19952 TITLE

From Page No. 5

Split all spinners + plate



To Page No. 7

Witnessed & Understood by me,	Date	Invented by	Date FRI
		Recorded by WM Bacon	10/22/93